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RELATIONSHIP OF STABLE COMBINATIONS OF SALIVARY CATECHOLAMINES WITH CEREBRAL FUNCTION ORGANIZATION IN PATIENTS WITH CHRONIC CEREBRAL ISCHEMIA

Fokin VF ✉, Abaimov DA, Ponomareva NV, Medvedev RB, Konovalov RN, Lagoda OV, Krotenkova MV, Shabalina AA, Tanashyan MM

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The study of the role of catecholamines (CAs) in cerebral organization of functions in patients with chronic cerebral ischemia (CCI) is relevant, since their important role as neurotransmitters is well known, along with the association with stress severity and cortisol. The study aimed to assess the impact of stable combinations of dopamine (DA), norepinephrine (NA), and adrenaline (ADR) on the organization of cerebral functions. A total of 76 patients with CCI were assessed based on the fMRI data (n = 21) converted into a network structure using the SPM-12 and CONN-18 software tools. Significance level estimation involved adjustment for multiple comparisons. Stable combinations of CAs reflecting mutual positive correlation of DA, NA and BP significantly affected cerebral organization of patients with CCI. CA combinations were associated with salivary cortisol ($F = 4.8$; $p = 0.038$) and memory ($F = 7.5$; $p = 0.011$) indices: the CA level increase was associated with increased cortisol levels and worse memory indices. Based on fMRI data the differences were revealed in connectivity organization of CCI patients with high and low levels of all three CAs. Patients with the CA content below median are characterized by the presence of closed neural networks extending to both brain hemispheres, which contributes to information integration and retention. It is assumed that such networks may be associated with the long-term potentiation mechanisms playing an important role in memory processes and changes in the synaptic connection strength. Thus, the use of non-invasive biochemistry testing methods and fMRI has made it possible to obtain new data on the ring organization of brain neural networks associated with stable CA combinations. Such neural network organization is likely to affect cognitive functions. High catecholamine levels in CCI patients are associated with increased cortisol levels, memory deterioration, and decreased connectivity in neural network of the brain.

Keywords: catecholamines, dopamine, norepinephrine, adrenaline, chronic cerebral ischemia, cerebral functions, connectivities, fMRI, neural networks

Author contribution: Fokin VF — manuscript writing; Abaimov DA — biochemistry testing; Ponomareva NV — physiological and neuropsychological testing design, general study design; Medvedev RB — Doppler tests and clinical assessment; Konovalov RN — brain imaging testing design; Lagoda OV — clinical assessment; Krotenkova MV — brain imaging testing management; Shabalina AA — biochemistry testing design; Tanashyan MM — clinical assessment management, general study design.

Compliance with ethical standards: the study was approved by the Ethics Committee of the Research Center of Neurology (protocol No. 5-6/22 dated 1 June 2022). The informed consent was submitted by all study participants.

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СВЯЗЬ УСТОЙЧИВЫХ СОЧЕТАНИЙ САЛИВАРНЫХ КАТЕХОЛАМИНОВ С ОРГАНИЗАЦИЕЙ ЦЕРЕБРАЛЬНЫХ ФУНКЦИЙ У БОЛЬНЫХ ХРОНИЧЕСКОЙ ИШЕМИЕЙ МОЗГА

В. Ф. Фокин ✉, Д. А. Абаимов, Н. В. Пономарева, Р. Б. Медведев, Р. Н. Коновалов, О. В. Лагода, М. В. Кротенкова, А. А. Шабалина, М. М. Танашян

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Исследование роли катехоламинов (КА) в церебральной организации функций у больных хронической ишемией мозга (ХИМ) актуально, поскольку известна их значительная роль как нейромедиаторов, а также связь с уровнем стресса и кортизолом. Целью работы было изучить влияние устойчивых сочетаний дофамина (ДА), норадреналина (НА) и адреналина (АДР) на организацию церебральных функций. Исследование выполнено на 76 больных ХИМ, с использованием данных фМРТ (n = 21), которые с помощью программ SPM-12 и CONN-18 преобразовывали в сетевую структуру. Оценку уровня значимости проводили с учетом множественности сравнений. Стабильные комбинации КА, отражающие взаимную положительную корреляцию ДА, НА и АДР, существенно влияли на церебральную организацию больных ХИМ. Комбинации КА были связаны с показателями саливарного кортизола ($F = 4,8$; $p = 0,038$) и памяти ($F = 7,5$; $p = 0,011$): повышение КА было ассоциировано с повышением кортизола и ухудшением показателей памяти. По данным фМРТ найдены различия в коннективной организации больных ХИМ с высоким и низким уровнем всех трех КА. Для пациентов с содержанием КА ниже значения медианы характерно наличие замкнутых нейронных сетей, распространяющихся на оба полушария головного мозга, что способствует интеграции и сохранению информации. Предполагается, что такие сети могут иметь связь с механизмами долговременной потенциации, играющими важную роль в процессах памяти и изменениями силы синаптической связи. Таким образом, использование неинвазивных методов биохимического анализа, а также фМРТ позволило получить новые данные о кольцевой организации нейросетей мозга, связанной с устойчивыми комбинациями КА. Подобная организация нейросетей, по-видимому, влияет на когнитивные функции. Высокий уровень катехоламинов у больных ХИМ связан с повышением уровня кортизола и ухудшением памяти и снижением коннективности нейросетей мозга.

Ключевые слова: катехоламины, дофамин, норадреналин, адреналин, хроническая ишемия мозга, церебральные функции, коннективности, фМРТ, нейросети

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Catecholamines (CAs) represent a group of bioactive substances playing an important and sometimes crucial role in regulation of various body's physiological processes. These belong to the class of monoamines and include three major compounds: dopamine (DA), norepinephrine (NA), and adrenaline (ADR). All CAs are synthesized from the amino acid tyrosine. The sequence of CA synthesis is as follows: tyrosine — dioxyphenylalanine (DOPA) — dopamine — noradrenaline — adrenaline. CAs are produced primarily by adrenal glands, as well as in the neurons of the central and peripheral nervous systems. The above CA synthesis pathway is referred to as classic or major CA synthesis pathway. Non-classic synthesis pathways (for example, from microbiome) are insufficient to have an effect on the CA content in the brain or body as a whole.

Common CA functions include regulation of mood and emotional state. Dopamine is often associated with the feeling of pleasure and reward. NA and ADR represent the key components of stress response. These alter cardiac output and heart rate, contribute to dilation of blood vessels in certain regions [1].

CA functions are related to their molecular organization: DA acts primarily on dopamine receptors, affecting motor functions, attention, and motivation. DA has an impact on the functions associated with reward, mood, and motion, playing a role in motor control.

NA is involved in stress responses and is in demand for increasing attention and alertness. It acts on the alpha and beta adrenergic receptors, contributing to blood pressure increase, heart rate increase, and better concentration. ADR triggers bronchial dilation, blood glucose level increase, and metabolic activity enhancement. ADR is responsible for acute stress reactions, ensuring rapid physical activity increase (heart rate increase, blood pressure elevation, and increased blood supply to muscles). One more important aspect associated with the CA common origin is the lack of negative correlations between distinct CAs and tyrosine. Some indirect negative regulation methods have been found, for example due to competition for common receptors, but the role of such regulation is minor. Such features of CA metabolism result in mostly positive correlations between DA, NA, and ADR [1, 2].

Salivary catecholamine levels may vary depending on various factors, such as time of the day, health status, fact of having stress, and sample collection method. A certain role is played by the method to determine the CA content. According to the literature data provided by different authors, the CA content varied between several pg/ml and tens of ng/ml [3–5]. Since salivary CAs are very variable, it seems reasonable to consider relative, mostly qualitative, but not quantitative indices.

Catecholamines are easily detected in human saliva, but the origin of those is poorly understood. The majority of papers report that CAs enter the salivary glands through the bloodstream. It has been shown that some CAs, for example NA found in human saliva, come both from the bloodstream and sympathetic nerves of the salivary glands [5].

The common source of CAs suggests mutual conditioning of those. However, the associated influence of catecholamines on brain function is still poorly understood. With mutual correlations of distinct CAs, resulting, in particular, from their common origin, a synchronized CA alteration can be observed [6]. In this regard, the study aimed to assess the impact of salivary CA combinations on connectivity of the brain neural networks, memory indices, and cortisol levels, when the quantity of all three CAs is above or below median, in patients with chronic cerebral ischemia (CCI), the disorder characterized by chronic disorder of cerebral circulation and the related diffuse or focal brain lesions, as well as cognitive and neurological deficits. The diagnosis of CCI is established based on the comprehensive assessment of clinical, instrumental, and neuropsychological data [7–10] (for details see Methods).

METHODS

The study involved 76 patients with CCI (42 females and 34 males aged 58–82 years). Salivary levels of catecholamines (DA, NA, ADR) were assessed. The patients were different from each other mostly in quantitative characteristics of memory impairment, performance, irritability, brainstem symptoms, etc. The main CCI etiological causes were as follows: atherosclerosis, hypertension (including hypertensive heart disease), venous insufficiency, diabetic angiopathy, vasculitis of various etiology, blood disorders, etc. Inclusion criteria: initial manifestations and subcompensated CCI; no need for permanent care from others in patients' daily life. Exclusion criteria: dementia severity score 1 or more (Clinical Dementia Rating) [11]; history of acute cerebrovascular accident, traumatic brain injury, severe cardiac or metabolic disorder (type 2 diabetes mellitus); renal failure, uncompensated thyroid dysfunction. All patients were right-handed.

The diagnosis of CCI was established based on the comprehensive assessment of clinical, instrumental, and neuropsychological data. Cognitive deficits represent the key sign of CCI often manifested by mild cognitive impairment. Assessment involved the use of neuropsychological tests: mini-mental state examination (MMSE) for general cognitive function assessment; Montreal Cognitive Assessment (MoCA test) more sensitive to mild impairment, especially affecting executive functions and attention; Luria's verbal memory tests.

Typical CCI symptoms were as follows: deterioration of memory, attention, decreased information processing speed, executive function (planning, decision making) impairment.

Structural MRI was focused on determining the white matter damage extent (leukoaraiosis). Fazekas score was used to estimate the damage severity.

MRI was used to detect lacunar infarctions, small foci (3–15 mm) in deep brain structures (basal ganglia, thalamus, pons). Mild-to-moderate ventricular dilation or cortical atrophy was one more CCI sign.

Neurological assessment revealed motor and sensory deficits typical for CCI: mild hemiparesis, dysmetria, reflex asymmetry, abnormal reflexes (for example, Babinski's sign); gait disorders; autonomic nervous system dysfunction, such as orthostatic hypotension; extrapyramidal symptoms, such as tremor.

Neuropsychological testing was used to clarify the nature and severity of cognitive impairment affecting memory (verbal, visual), executive functions, speech activity, visual-spatial abilities.

Differential diagnosis with neurodegenerative disorders took place.

fMRI

A total of 21 patients underwent T2* weighted resting state fMRI of the brain in order to record BOLD signal in the Magnetom Verio magnetic resonance imaging scanner (Siemens, Germany) with the magnetic field strength of 3.0 Tesla. Functional scans were acquired in the resting state using the T2* weighted EPI sequence: TR = 1500 ms, TE = 30 ms, flip angle 70°, slice thickness 2 mm, FOV 190 mm, FOV phase 100.0%. The patients were previously instructed to relax as much as possible, lay still with the eyes closed (to avoid stimulation of visual sensory system), not to think about anything in particular. MRI data were processed using the SPM12 software in the MATLAB computing environment. The CONN-18b application being part of the SPM-12 toolbox was used to assess connectivity.

Biochemical tests

Determination of salivary catecholamines. Salivary levels of monoamines, including DA, NA, ADR, and their metabolites were determined by high performance liquid chromatography (ion pair chromatography) with electrochemical detection (HPLC-ED) using the System Gold liquid chromatography system (Beckman Coulter Inc., USA) equipped with the RECIPE EC 3000 electrochemical (amperometric) detector (RECIPE Chemicals + Instruments GmbH; Germany), with the Rheodyne 7125 injector, 20 µL sample loop. Catecholamines were separated by chromatography on the reverse-phase Nucleodur C18 Gravity column, 4.6–250 mm, pore diameter 5 µm (Mashery-Nagel GmbH & Co. KG, Germany). The System Gold 125 pump (Beckman Coulter Inc., USA) was used; the mobile phase flow rate was 1 mL/min at the pressure of 200 atm. atm. The mobile phase for catecholamine separation was as follows: 0.1 M citrate-phosphate buffer containing 1.1 mM of octanesulfonic acid, 0.1 mM of EDTA, and 9% of acetonitrile (pH 3.0). Measurement was performed using the RECIPE EC 3000 electrochemical detector (RECIPE Chemicals + Instruments GmbH; Germany) equipped with the ClinLab ECD-Cell, Model Sputnik, glassy carbon working electrode (+ 0.85 V), and silver chloride reference electrode Ag/AgCl. Prior to chromatography, catecholamines were isolated from saliva by solid phase extraction using the activated aluminum oxide as an extractant.

The patients' salivary cortisol levels were determined with the Abbott i2000 ARCHITECT immunochemiluminescence analyzer (Abbott Laboratories, Illinois, USA) using the reagent kits of the same brand.

Saliva samples were collected in accordance with the previously reported protocol [9]. The patients did not drink alcohol for a week, tea or coffee for 1 h before saliva collection; they rinsed their mouth with water 10 min before this. Saliva collection was accomplished through spitting into a test tube with the volume of at least 1.5 mL. Saliva samples contaminated with blood were excluded from the study. For that the ELISA kit for detection of saliva contamination with blood was used [12].

Other tests

The patients were tested for verbal memory using the Luria's test involving control of immediate and delayed recall of 10 words. They were through verbal fluency test, correction test. Furthermore, blood pressure was recorded, pulse pressure (difference between systolic and diastolic blood pressure) was calculated, and heart rate was registered.

Statistical analysis

The data obtained were analyzed using the Statistica-12 software package (Dell, USA). The distribution was tested for normality using the Kolmogorov–Smirnov test and Lilliefors test. The mean and standard error were calculated, and one-way analysis of variance and correlation analysis were performed. Spearman's rank correlation coefficient was calculated. To analyze neural networks, Student's *t*-test was also calculated, and adjustment for multiple comparisons was applied — FDR (False Discovery Rate).

RESULTS

CA content distribution: the salivary DA, NA, and ADR distribution associated with chronic cerebral ischemia was significantly different from normal (Gaussian) distribution (Fig. 1).

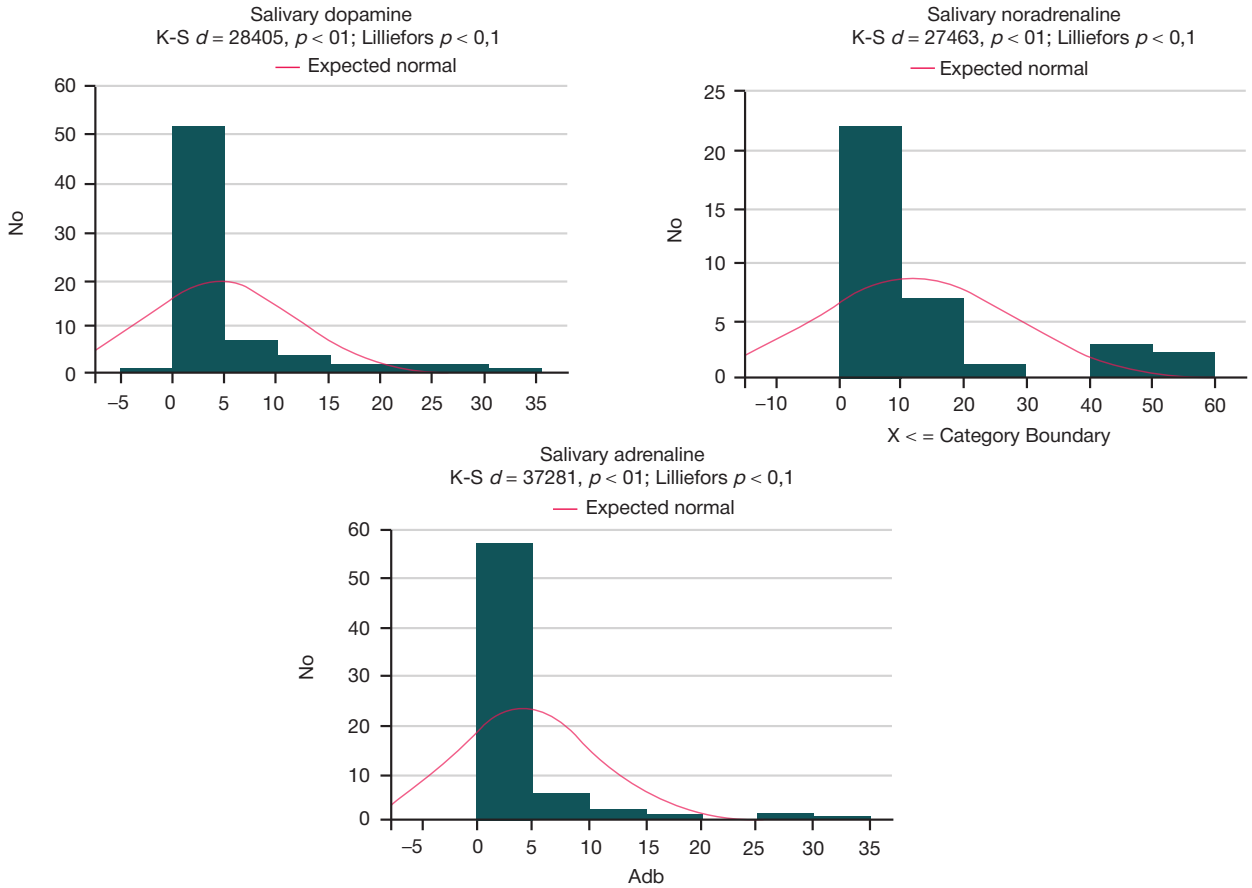

Fig. 1. Bar charts of salivary dopamine, norepinephrine, and adrenaline distribution (ng/mL). Vertical axis (Y) — number of subjects; horizontal axis (X) — catecholamine concentration (ng/mL). K-S — Kolmogorov–Smirnov test, Liliefors — Lilliefors test

Table 1. Spearman's rank correlation (R)

Pair of variables	Number	Spearman's rank correlation coefficient(R)	t(N-2)	*p*-value
NA & ADR	76	0.511801	5.12474	0.000002
NA & DA	65	0.509447	4.699123	0.000015
ADR & DA	62	0.490695	4.362181	0.000051

Note: NA — norepinephrine; ADR — adrenaline; DA — dopamine; R — Spearman's rank correlation coefficient; t — Student's *t*-test; *p* — significance level.

The Kolmogorov–Smirnov test and Lilliefors test values suggest significant difference of experimental distributions from normal (Gaussian) distribution. Fig. 1 presents bar charts of the distribution of salivary dopamine, norepinephrine, and adrenaline. The range of changes of these monoamines is consistent with the above literature data [3–6].

Distribution of various catecholamines in saliva was generally close to log-normal. For testing, logarithms of all baseline values were taken. It was found that based on the Kolmogorov–Smirnov test d (maximum difference between the theoretical and empirical distributions) was as follows: for NA $d = 0.069$; for ADR $d = 0.086$; for DA $d = 0.092$. P-values > 0.2 obtained for these logarithmic distributions suggest that there is no reason to believe that these logarithmic distributions are different from normal for the test CAs.

Due to the common origin, contingency of catecholamines between pairs of CAs in the background is not surprising. Table 1 presents the correlation coefficient values obtained when using nonparametric rank statistics.

The use of Spearman's rank correlation analysis revealed a moderate positive correlation between all CA pairs. All the rank correlation coefficients were significantly different from zero (Table 1). The squared correlation coefficient demonstrates the share of the influence or factors explaining variation of a single variable through the relationship with another one, since it shows what proportion of the overall variability of a variable is due to the linear relationship with another variable. That is why the share of factors determined by joint effects of CAs on each other is about 25%. Such contingency suggests that in patients with chronic cerebral ischemia the probability of having high (or low) levels of all three catecholamines at once is higher, than the probability of having any other inconsistent combination of CA levels.

All patients were divided into two subgroups: in each subgroup, CA content was above or below median.

The share of cases, when the levels of all three CAs were above or below median, was slightly more than a half (28 cases out of 50 possible). This means that the conditions, when all three CAs at once are above or below median levels, account for at list a half of all possible variants. The median value for dopamine was 1.447 (min — 0; max — 30.341) ng/mL; for norepinephrine — 5.577 (min — 0.954; max — 56.647) ng/mL; for adrenaline — 2.408 (min — 0.057; max — 90.257) ng/mL.

The number of patients with the levels of all three CAs below median was 13 (group 1), and that of patients with the levels of all three CAs above median was 15 (group 2).

Groups 1 and 2 differed from each other in that the levels of all three CAs were below or above median. Patients of these groups were significantly different based on the number of psychophysiological characteristics. Thus, patients of group 1 had significantly lower cortisol levels, and their delayed word recall scores (Luria's test) were better, than that of group 2 representatives (Fig. 2).

Such differences in psychophysiological characteristics of two groups support the idea that there are differences in cerebral organization of these patients. To estimate the differences in cerebral organization of groups 1 and 2, we assessed connectivity difference in the groups with the levels of all three CAs below or above median: group 1 — group 2.

Considering False Discovery Rate (FDR), all the connectivities, that were different with lower CA levels, were significantly higher, than with higher CA levels (Fig. 3).

We chose not the conventional pFDR < 0.05, but the lower significance level pFDR < 0.02, to consider more significant patterns, in which the plethora of connectivities of two groups were different (Fig. 3).

Thanks to the cyclic (closed) organization of the connections (Fig. 3) most common in group 1 with the lower CA levels, stable excitation circulation is generated engaging the large

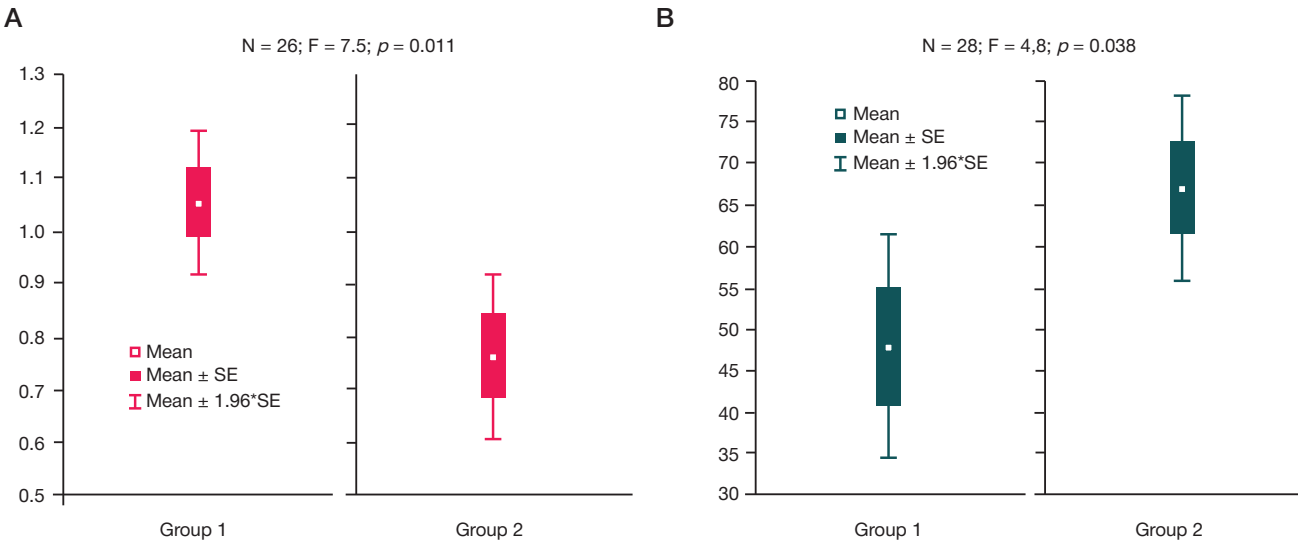

Fig. 2. Differences in memory characteristics **(A)** and salivary cortisol levels **(B)** in two groups with different CA values. N — number of subjects; F — F-test; *p* — significance level. **A**. The vertical axis shows delayed word recall relative to the average level of direct recall of 10 words (Luria's test). **B**. The vertical axis shows salivary cortisol levels (nmol/L)

number of neural centers, which contributes to more optimal functional organization of the brain, as also confirmed by the results of psychophysiological tests (Fig. 2).

Circulation of excitation is possible, for example, in the following chain of the neural structures linked with the common excitation process synergy:

Cerebellum (including the Vermis) — right lateral sensotimotor network (Sensori Motor Lateral) — left superior frontal gyrus (SFG) — left supramarginal gyrus (SMG) — cerebellum. This circuit engaging both hemispheres can maintain generalized synchronization of excitation processes.

DISCUSSION

In our studies, salivary cortisol and catecholamine levels were determined by biochemical methods. According to the literature data, salivary levels of these substances in individuals with CCI are considerably higher, than in healthy individuals. In the morning, salivary cortisol levels usually reach a few to tens of nmol/L (the levels of about ~10–20 nmol/L are often reported in the literature). Higher values are reported for CCI. In this study, cortisol levels associated with CCI were about 3–3.5 times higher compared to normal based on the literature data [12–14].

In CCI, an upward trend of salivary catecholamine levels is reported, which reflects enhancement of sympathetic activity and impaired vascular regulation. However, measurement in saliva requires standardization for clinical use. The difficulty is that catecholamine measurement has not been standardized, and only a few authors report exact numbers: normal salivary noradrenaline are 20–30 pg/mL, and adrenaline levels are approximately 3–4 pg/mL [5]. Another paper reports rough equivalence of adrenaline and norepinephrine (0.1 pmol/L) [4], and dopamine level is approximately 0.5 pmol/L [4–5].

Traditionally, assessment of distinct CAs is often considered without taking into account their association with other ones. Such an approach has the following shortcoming: since CAs are related due to common origin, it is rather difficult to isolate the features of distinct CAs, and in some cases these should be considered in combination with other CAs. The CA activity largely reflects the functional state of the brain, for example, due to positive correlation between the levels of some CAs (ADR and NA) and cortisol levels. One variant of the “contextual” consideration is the above analysis of the synchronized CA states. Such functional states affect both cognitive and metabolic indices, especially the ones associated with stress severity. The range of CA changes is different on both sides of the median. This is probably due to the fact that CA production is altered under exposure to stress, and dysfunction of the negative feedback mechanism limiting the release of CAs is possible, which can also be due to stress [15, 16]. In healthy individuals, the quantity of CAs released by the adrenal glands and sympathetic nervous system is regulated by the negative feedback mechanisms. When a certain CA level is reached, further CA production is inhibited, which prevents excess activation of the sympathoadrenal system. In prolonged or chronic stress, permanent stimulation of the sympathetic nervous system and adrenal glands is observed. This results in the permanently high blood catecholamine levels. The negative feedback mechanism begins to fail, i.e. the control over adrenaline and norepinephrine production is lost, which leads to negative effects on the brain function.

The main distinctive feature of the brain's connective organization in the group with lower CA levels is the presence of the closed circuit of connectivities, which can result in the prolonged potentiation processes. Closed neural networks are networks, in which inputs and outputs represent the closed cycle, ensuring information storage and processing within the closed structure [17]. The neural networks of the patients different in salivary NA levels only were earlier considered; these networks were not cyclic (closed) [18].

It can be assumed that excitation circulation in the closed circuit of neural structures can really maintain synergetic processes between various brain regions. Consider some characteristic features of this circuit.

It is well known, that the cerebellum plays a key role in motor coordination and maintaining balance. It is also involved in cognitive functions, such as learning and memory. Its capability of integrating sensory information and motor output makes it an important link of this circuit. The cerebellum also can integrate information from various sensorimotor sources, ensuring coordination between various body parts and modulating the activity of cortical structures. The link between the right lateral sensorimotor network and the left cortical structures (superior frontal gyrus and supramarginal gyrus) can be associated with the mechanisms underlying cross-modal sensory processing, enabling processing and integration of information from both sides of the body and sensory inputs. Furthermore, the left superior frontal (SFG) and supramarginal (SMG) gyri are involved in higher cognitive functions, such as attention, problem-solving, and planning. Excitation increase in these regions can enhance cognitive information processing and improve the controlled actions related to motor activity. The above chain can effectively use the feedback mechanisms. Excitation initiated in one node can be returned to the previous nodes to ensure stable activity and possible enhancement (potentiation)

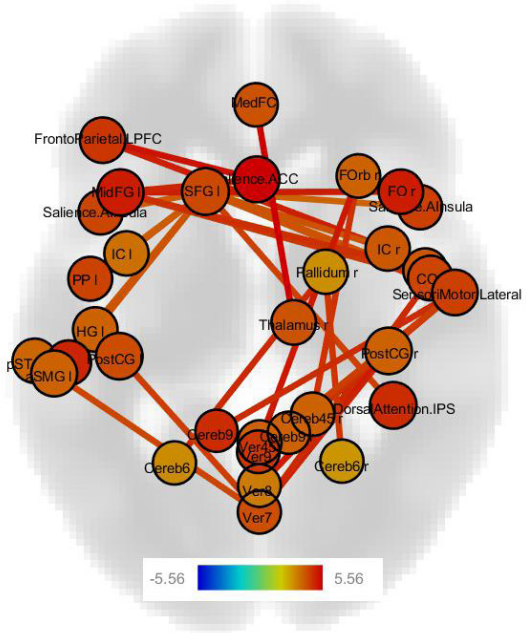

Fig. 3. Connectivity difference with the more low and high levels of all catecholamines (group 1 — group 2), pFDR < 0.02. There is a Student’s t-test color chart below the figure. r, l — right and left hemispheres; Ver — Vermis, Cereb — cerebellum; digits following Ver, Cereb — share of the vermis or cerebellum; AC — Cingulate Gyrus anterior part; ACC — Anterior Cingulate Cortex; Cuneal — Cuneal Cortex; CO — Center Operculum Cortex; Forb — Frontal Orbital Cortex; HG — Heschl’s Gyrus; IC — Insular Cortex; sLOC — Lateral Occipital Cortex superior division; MedFC — Frontal Medial Cortex; PC — Cingulate Gyrus posterior division; aPaHC — Parahippocampal Gyrus anterior division; PaCiG — Paracingulate Gyrus; PostCG — Postcentral Gyrus; PreCG — Precentral Gyrus; PP — Planum Polare; SMA — Juxtapositional Lobule; SFG — Superior Frontal Gyrus; aSMG — aSTG — Superior Temporal Gyrus anterior division; toITG — Inferior Temporal Gyrus temperooccipital division; toMTG — Middle Temporal Gyrus temporooccipital part

of synaptic strength in these areas. Such a “closed contour” can contribute to the neuronal activity synchronization between various brain regions, which is important for maintaining behavior stability and motor coordination. Excitation circulation can contribute to alteration of structure at the synaptic level, since repeated activation can result in the processes related to synaptic plasticity (such as long-term potentiation and long-term depression) that can strengthen the connections between appropriate neuronal structures.

Thus, excitation circulation in the above neural circuit demonstrates a complex interplay between motor coordination and cognitive processing. Such interplay can maintain effective functioning in both sensorimotor and cognitive spheres, ensuring a synergistic mechanism for adaptation and learning. In the closed circuits of connectivities, such as neural networks, excitation can circulate, generating repeated launches of neuronal activity. This process can contribute to the long-term potentiation through the following mechanisms.

1. Enhanced neuronal activation. Prolonged excitation circulation can maintain high intracellular calcium levels in the neurons. The increase in calcium levels contributes to more potent release of neurotransmitters and enhances synaptic transmission being the most important component of both short-term and long-term synaptic plasticity [19].

2. Creating positive feedback. In the closed circuit, excitation can result in active return of the signal through the excitatory synapses. Such a mechanism can yield the state contributing to potentiation, since the neurons connected are still activated, which enhances transmission of information and prepares synapses for further activation [20].

3. Neuronal activity modulation. Mutual modulation of neurons in the circuit can result in the long-term synaptic strength modulation, which provides the basis for synaptic plasticity. This phenomenon, in turn, contributes to the long-term potentiation [21]. Synaptic strength is a measure of the effectiveness of signal transmission between neurons at the synaptic level. Synaptic strength determined by how much a postsynaptic neuron responds to the presynaptic neuron activation reflects the amount of neurotransmitters released into the synaptic cleft, as well as the sensitivity of postsynaptic receptors to these neurotransmitters.

Potentiation can be modulated via different mechanisms, such as upregulation of receptors or synaptic structure alteration, which can consolidate changes in the neural network related to the learning and memory processes.

Thus, it can be assumed, that prolonged excitation circulation in the closed circuit can maintain the conditions for long-term potentiation. This is especially important in the context of neuroplasticity, learning and memory processes, since alteration of synaptic transmission can result in the lasting neural network alteration.

However, elevated cortisol and catecholamine levels disturb blood supply to the brain and cause cessation of excitation circulation in the neural networks of the brain [22].

CONCLUSIONS

We have revealed a significant correlation between stable salivary catecholamine combinations and organization of cerebral functions in patients with chronic cerebral ischemia. The findings confirm that comprehensive simultaneous analysis of salivary DA, NA, and ADR levels can be an informative marker of the brain functional state associated with this disorder. In the group of patients with CCI showing high contingency of salivary CAs with the relatively low CA levels (below median), the presence of closed neural networks was reported. It is assumed that such networks can contribute to higher long-term potentiation, possibly through which this group of patients has higher cognitive indices. High catecholamine and cortisol levels associated with disturbances of blood supply to the brain negatively affect connectivity of the brain neural networks. The noninvasive method to assess catecholamines and the qualitative analysis of those can be useful for investigation of the brain network organization associated with the cerebrovascular disease.

References

1. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacol Rev. 2004; 56 (3): 331–49. DOI: 10.1124/pr.56.3.1. PMID: 15317907.
2. Goldstein DS, Eisenhofer G, Kopin, IJ. Sources and Significance of Plasma Levels of Catechols and Their Metabolites in Humans. Journal of Pharmacology and Experimental Therapeutics. 2003; 305 (3), 800–811. Available from: https://doi.org/10.1124/JPET.103.049270.
3. Schwab KO, Heubel G, Bartels H. Free epinephrine, norepinephrine and dopamine in saliva and plasma of healthy adults. Eur J Clin Chem Clin Biochem. 1992; 30 (9): 541–44. PMID: 1457617.
4. Okumura T, Nakajima Y, Matsuoka M, Takamatsu T. J Chromatogr B Biomed Sci Appl. 1997; 694 (2): 305–16. DOI: 10.1016/s0378-4347(97)00106-0. PMID: 9252044.
5. Kennedy B, Dillon E, Mills PJ, Ziegler MG. Catecholamines in human saliva. Life Sci. 2001; 69 (1): 87–99. DOI: 10.1016/s0024-3205(01)01111-0. PMID: 11411808.
6. Lei, S. (2014). Cross interaction of dopaminergic and adrenergic systems in neural modulation. International Journal of Physiology, Pathophysiology and Pharmacology. 2014; 6 (3): 137–42. Available from: https://pubmed.ncbi.nlm.nih.gov/25349636/.
7. Parfenov VA. Sosudistye kognitivnye narusheniya i hronicheskaya ishemiya golovnogo mozga (discirkulyatornaya encefalopatiya). Nevrologiya, nejropsihiatriya, psihosomatika. 2019; 11 (Pril. 3): 61–67. Russian.
8. Tanashyan MM, Antonova KV, Raskurazhev AA, Lagoda OV. Cerebrometabolicheskoe zdorov'e. M.: MediaSfera, 2025; 260 s.
9. Graff-Radford J. Vascular Cognitive Impairment. Continuum (Minneap Minn). 2019; 25 (1): 147–64. DOI: 10.1212/CON.0000000000000684. PMID: 30707191; PMCID: PMC6548535.
10. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al. Standards for ReportIng Vascular changes on nEuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013; 12 (8): 822–38. DOI: 10.1016/S1474-4422(13)70124-8. PMID: 23867200; PMCID: PMC3714437.
11. Morris JC. Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. Int Psychogeriatric. 1997; (9 Suppl 1): 173–76.
12. Vining RF, McGinley RA, Maksvytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. Ann Clin Biochem. 1983; 20 (Pt 6): 329–35. DOI: 10.1177/000456328302000601. PMID: 6316831.
13. Kudielka BM, Kirschbaum C. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. Psychoneuroendocrinology. 2003; 28 (1): 35–47. DOI: 10.1016/s0306-4530(02)00008-2. PMID: 12445835.
14. Fokin VF, SHabalina AA, Ponomareva NV, Medvedev RB, Lagoda OV, Tanashyan MM. Kortizol, citokiny i vegetativnye izmeneniya pri kognitivnoj nagruzke u bol'nyh hronicheskoj ishemiej mozga.

Patologicheskaya fiziologiya i eksperimental'naya terapiya. 2023; 67 (3): 51–57. DOI: 10.25557/0031-2991.2023.03.51-57. Russian.
15. Kvetnansky R, Sabban EL, Palkovits M. Catecholaminergic systems in stress: structural and molecular genetic approaches. Physiol Rev. 2009; 89 (2): 535–606. DOI: 10.1152/physrev.00042.2006. PMID: 19342614.
16. Goldstein DS. Catecholamines and stress. Endocrine Regulations. 2003; 37 (2): 69–80. Available from: https://pubmed.ncbi.nlm.nih.gov/12932192/.
17. Rosenbaum R. Short Term Plasticity, Biophysical Models. Available from: https://dblp.uni-trier.de/db/reference/cn/cn2014.html.
18. Fokin VF, Abaimov DA, Ponomareva NV, Medvedev RB, Konovalov RN, Lagoda OV, i dr. Nejroseti, chuvstvitel'nye k urovnyu noradrenalina u bol'nyh hronicheskoj ishemiej mozga. Asimmetriya. 2022; 16 (3): 23–31. Russian.
19. Zucker RS, Regehr WG. Short-term synaptic plasticity. Annu Rev Physiol. 2002; 64: 355–405. DOI: 10.1146/annurev.physiol.64.092501.114547. PMID: 11826273.
20. Thompson RF, Kim JJ. Memory systems in the brain and localization of a memory. Proc Natl Acad Sci USA. 1996; 93 (24): 13438–44. DOI: 10.1073/pnas.93.24.13438. PMID: 8942954; PMCID: PMC33628.
21. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron. 2004; 44 (1): 5–21. DOI: 10.1016/j.neuron.2004.09.012. PMID: 15450156.
22. Tank AW, Lee Wong D. Peripheral and central effects of circulating catecholamines. Compr Physiol. 2015; 5 (1): 1–15. DOI: 10.1002/cphy.c140007.

Литература

1. Eisenhofer G, Kopin IJ, Goldstein DS. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacol Rev. 2004; 56 (3): 331–49. DOI: 10.1124/pr.56.3.1. PMID: 15317907.
2. Goldstein DS, Eisenhofer G, Kopin, IJ. Sources and Significance of Plasma Levels of Catechols and Their Metabolites in Humans. Journal of Pharmacology and Experimental Therapeutics. 2003; 305 (3), 800–811. Available from: https://doi.org/10.1124/JPET.103.049270.
3. Schwab KO, Heubel G, Bartels H. Free epinephrine, norepinephrine and dopamine in saliva and plasma of healthy adults. Eur J Clin Chem Clin Biochem. 1992; 30 (9): 541–44. PMID: 1457617.
4. Okumura T, Nakajima Y, Matsuoka M, Takamatsu T. J Chromatogr B Biomed Sci Appl. 1997; 694 (2): 305–16. DOI: 10.1016/s0378-4347(97)00106-0. PMID: 9252044.
5. Kennedy B, Dillon E, Mills PJ, Ziegler MG. Catecholamines in human saliva. Life Sci. 2001; 69 (1): 87–99. DOI: 10.1016/s0024-3205(01)01111-0. PMID: 11411808.
6. Lei, S. (2014). Cross interaction of dopaminergic and adrenergic systems in neural modulation. International Journal of Physiology, Pathophysiology and Pharmacology. 2014; 6 (3): 137–42. Available from: https://pubmed.ncbi.nlm.nih.gov/25349636/.
7. Парфенов В. А. Сосудистые когнитивные нарушения и хроническая ишемия головного мозга (дисциркуляторная энцефалопатия). Неврология, нейропсихиатрия, психосоматика. 2019; 11 (Прил. 3): 61–67.
8. Танашян М. М., Антонова К. В., Раскуражев А. А., Лагода О. В. Цереброметаболическое здоровье. М.: МедиаСфера, 2025; 260 с.
9. Graff-Radford J. Vascular Cognitive Impairment. Continuum (Minneap Minn). 2019; 25 (1): 147–64. DOI: 10.1212/CON.0000000000000684. PMID: 30707191; PMCID: PMC6548535.
10. Wardlaw JM, Smith EE, Biessels GJ, Cordonnier C, Fazekas F, Frayne R, et al. Standards for ReportIng Vascular changes on nEuroimaging (STRIVE v1). Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. Lancet Neurol. 2013; 12 (8): 822–38. DOI: 10.1016/S1474-4422(13)70124-8. PMID: 23867200; PMCID: PMC3714437.
11. Morris JC. Clinical dementia rating: a reliable and valid diagnostic and staging measure for dementia of the Alzheimer type. Int Psychogeriatric. 1997; (9 Suppl 1): 173–76.
12. Vining RF, McGinley RA, Maksvytis JJ, Ho KY. Salivary cortisol: a better measure of adrenal cortical function than serum cortisol. Ann Clin Biochem. 1983; 20 (Pt 6): 329–35. DOI: 10.1177/000456328302000601. PMID: 6316831.
13. Kudielka BM, Kirschbaum C. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. Psychoneuroendocrinology. 2003; 28 (1): 35–47. DOI: 10.1016/s0306-4530(02)00008-2. PMID: 12445835.
14. Фокин В. Ф., Шабалина А. А., Пономарева Н. В., Медведев Р. Б., Лагода О. В., Танашян М. М. Кортизол, цитокины и вегетативные изменения при когнитивной нагрузке у больных хронической ишемией мозга. Патологическая физиология и экспериментальная терапия. 2023; 67 (3): 51–57. DOI: 10.25557/0031-2991.2023.03.51-57.
15. Kvetnansky R, Sabban EL, Palkovits M. Catecholaminergic systems in stress: structural and molecular genetic approaches. Physiol Rev. 2009; 89 (2): 535–606. DOI: 10.1152/physrev.00042.2006. PMID: 19342614.
16. Goldstein DS. Catecholamines and stress. Endocrine Regulations. 2003; 37 (2): 69–80. Available from: https://pubmed.ncbi.nlm.nih.gov/12932192/.
17. Rosenbaum R. Short Term Plasticity, Biophysical Models. Available from: https://dblp.uni-trier.de/db/reference/cn/cn2014.html.
18. Фокин В. Ф., Абаимов Д. А., Пономарева Н. В., Медведев Р. Б., Коновалов Р. Н., Лагода ОВ., и др. Нейросети, чувствительные к уровню норадреналина у больных хронической ишемией мозга. Асимметрия. 2022; 16 (3): 23–31.
19. Zucker RS, Regehr WG. Short-term synaptic plasticity. Annu Rev Physiol. 2002; 64: 355–405. DOI: 10.1146/annurev.physiol.64.092501.114547. PMID: 11826273.
20. Thompson RF, Kim JJ. Memory systems in the brain and localization of a memory. Proc Natl Acad Sci USA. 1996; 93 (24): 13438–44. DOI: 10.1073/pnas.93.24.13438. PMID: 8942954; PMCID: PMC33628.
21. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. Neuron. 2004; 44 (1): 5–21. DOI: 10.1016/j.neuron.2004.09.012. PMID: 15450156.
22. Tank AW, Lee Wong D. Peripheral and central effects of circulating catecholamines. Compr Physiol. 2015; 5 (1): 1–15. DOI: 10.1002/cphy.c140007.

EFFICACY OF USING MELATONIN PER RECTUM FOR EXPERIMENTAL ACUTE CEREBRAL ISCHEMIA

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With limited efficacy and safety of the methods to treat ischemic stroke (IS), melatonin (MT) can be considered a promising neuroprotective agent having a pleiotropic mechanism of action. The study aimed to assess the effect of MT contained in original rectal suppositories on the neurological status and microcirculation in the injury focus in experimental acute cerebral ischemia (EACI) *in vivo*. A total of 30 sexually mature rats were divided into three groups, 10 animals per group: sham-operated (SO) animals; animals with EACI; animals with EACI receiving original rectal suppositories weighing 100 mg with 2.5 mg of melatonin (MT) throughout 7 days. On days 3 and 7, neurological status was assessed using the Garcia JH score, Placing test, Bederson test; microcirculation rate (MR) was assessed in the brain injury focus by laser flowmetry. A significant decrease in the Garcia JH scores by 58.3% ($p = 0.001$), Placing Test scores by 57.9% ($p = 0.002$), along with the significant increase in the Bederson Test scores in animals with EACI compared to SO animals was reported on day 3; the significant decrease in the Garcia JH scores by 75% ($p < 0.001$), Placing Test scores by 78.9% ($p < 0.001$) and the significant increase in the Bederson Test scores were reported on day 7. MR decreased by 30% on day 3 ($p = 0.02$), by 38% on day 7 ($p = 0.005$). The use of the MT-based rectal suppositories resulted in the neurological deficit restoration in the form of the significant increase in the Garcia JH scores by 53.3% ($p = 0.008$), Placing Test scores by 50% ($p = 0.016$) and the significant decrease in the Bederson Test scores by 50% ($p = 0.029$) on day 3; on day 7, the significant increase in the Garcia JH scores by 233% ($p < 0.0001$), Placing Test scores by 325% ($p < 0.0001$) and the significant decrease in the Bederson Test scores by 100% ($p < 0.0001$) were reported. MR increased by 12.5% on day 3 ($p = 0.016$), by 43.9% on day 7 ($p = 0.005$). The correlation analysis revealed the association between the neurological status and MR values: the neurological deficit improvement in animals with EACI in the context of receiving the MT-based rectal suppositories was associated with the MR increase in the ischemic focus in the brain. Thus, partial neurological status restoration in the context of using the MT-based rectal suppositories for EACI resulted from the MT vasoactive properties, which was reflected in the MR increase in the ischemic focus in the brain.

Keywords: melatonin, rectal suppositories, ischemic stroke

Author contribution: Osikov MV — study concept and design, manuscript editing and approval; Shelomentsev AV — data acquisition, literature review, analysis and interpretation of the results; Boyko MS — statistical analysis; Shishkova YuS, Fedosov AA — manuscript editing.

Compliance with ethical standards: the study was approved by the Ethics Committee of the South Ural State Medical University (protocol No. 5 dated 10 June 2024). Animals were kept and handled in accordance with the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and animal handling principles based on the provisions of the Declaration of Helsinki and the guidelines contained in the EU Council Directive 86/609/ECC and the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

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ЭФФЕКТИВНОСТЬ ПРИМЕНЕНИЯ МЕЛАТОНИНА PER RECTUM ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ОСТРОЙ ИШЕМИИ ГОЛОВНОГО МОЗГА

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При лечении ишемического инсульта (ИИ) мелатонин (МТ) может быть перспективным нейропротектором с плейотропным механизмом действия. Цель исследования — *in vivo* изучить влияние МТ в составе оригинальных ректальных суппозиториев (РС) на неврологический статус (НС) и микроциркуляцию в очаге повреждения при экспериментальной острой ишемии головного мозга (ЭОИГМ). 30 крыс разделили на три группы по 10 особей: 1) ложнооперированные (ЛО); 2) особи с ЭОИГМ; 3) особи с ЭОИГМ, получающие оригинальные РС с МТ на протяжении 7 суток. Животным групп 2 и 3 моделировали ЭОИГМ по модифицированной методике Chen S. T., et al. На 3 и 7 сутки оценивали НС по шкалам Garcia J. H., Placing test, Bederson test и показатель микроциркуляции (ПМ) в очаге повреждения головного мозга методом лазерной флуометрии. У животных с ЭОИГМ по сравнению с ЛО на 3 сутки зафиксировано значимое снижение баллов по шкале Garcia на 58,3% ($p = 0,001$), по Placing test — на 57,9% ($p = 0,002$), увеличение баллов по Bederson test; на 7 сутки зафиксировано значимое снижение баллов по шкале Garcia J. H. на 75% ($p < 0,001$), по Placing test — на 78,9% ($p < 0,001$), увеличение баллов по Bederson test. ПМ на 3 сутки снизился на 30% ($p = 0,02$), на 7 сутки — на 38% ($p = 0,005$). Применение РС с МТ приводило к восстановлению неврологического дефицита в виде значимого увеличения баллов на 3 сутки по шкале Garcia на 53,3% ($p = 0,008$), по Placing test — на 50% ($p = 0,016$) и снижения баллов по Bederson test на 50% ($p = 0,029$); на 7 сутки фиксировали значимое увеличение баллов по шкале Garcia J. H. на 233% ($p < 0,0001$), по Placing test — на 325% ($p < 0,0001$) и снижение баллов по Bederson test на 100% ($p < 0,0001$). ПМ на 3 сутки повысился на 12,5% ($p = 0,016$), на 7 сутки на 43,9% ($p = 0,005$). Установлено, что уменьшение неврологического дефицита у животных с ЭОИГМ в условиях применения РС с МТ ассоциировано с повышением ПМ в очаге ишемического повреждения головного мозга. Таким образом, частичное восстановление НС в условиях применения РС с МТ при ЭОИГМ обусловлено его вазоактивными свойствами.

Ключевые слова: мелатонин, ректальные суппозитории, ишемический инсульт

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Today, ischemic stroke (IS) ranks second among the causes of death and third among the causes of disability all over the world [1]. More than 450,000 cases of stroke are reported annually in the RF, and case fatality rate varies between 17.6 and 20.7% [2]. The IS pathogenesis is multifaceted; it involves such mechanisms, as neuroinflammation, glutamate-induced excitotoxicity, and oxidative stress resulting in the death of neurons in the ischemic focus [3]. The existing pathogenetic approaches to treatment of IS show limited efficacy and safety due to narrow therapeutic window, high risk of hemorrhagic transformation, low permeability of the blood-brain barrier (BBB) for neuroprotective agents, as well as poor knowledge about their pharmacokinetics and rather frequent adverse effects, which emphasizes the need to develop new therapeutic strategies aimed at neuroprotection [4]. In this regard, melatonin (MT) possessing pleiotropic effects, including antioxidant, anti-inflammatory, vasoactive, anti-apoptotic ones, is of interest [5–7]. A number of papers demonstrate the MT neuroprotective effect in IS realized through its direct binding to free radicals, increase in activity of key antioxidant enzymes (glutathione peroxidase, catalase), inhibition of caspase-3 and NF-κB pathway, as well as the decrease in expression of AQP4 and enhanced SIRT-1 synthesis in the ischemic brain lesion and, therefore, cerebral infarction volume decrease with subsequent neurological deficit improvement [8–10]. However, the use of MT as a potential neuroprotective agent in oral dosage forms can be considerably limited by post-stroke dysphagia reported in 81% of cases in patients post IS [11]. The use of injectable dosage forms as an alternative allows one to work around the issue of post-stroke dysphagia, but it is associated with constant re-traumatization of the patient when performing injections, high risk of microbial contamination, etc. [12]. The MT-based rectal suppositories represent a promising dosage form ensuring the atraumatic administration and low risk of microbial contamination. In the RF, there are no approved MT-based rectal dosage forms for neuroprotective therapy of IS that could effectively deliver the drug and have a systemic effect on the ischemic injury focus in the brain, thereby minimizing the risk of injury and infectious complications. The development and assessment of the use of MT-based rectal suppositories in IS are relevant due to potential benefits of atraumatic administration routes. The study aimed to perform *in vivo* experimental assessment of the effect of melatonin contained in original rectal suppositories on the neurological status and microcirculation in the ischemic brain injury focus in experimental acute cerebral ischemia.

METHODS

The experiment involved 30 sexually mature male Wistar rats weighting 220–240 g obtained from the experimental biology clinic of the South Ural State Medical University of the Ministry of Health of the Russian Federation in spring and summer. The animals were kept with natural light, at a temperature of 20–22 °C and relative humidity of 60–70%. Simple randomization was used to divide the rats into three groups, 10 animals per group: group 1 — sham-operated (SO) animals, group 2 — animals with experimental acute cerebral ischemia (EACI), group 3 — animals with EACI receiving original rectal suppositories weighing 100 mg with 2.5 mg of MT every 24 h throughout 7 days [13].

EACI was simulated using the modified method by Chen ST under the combination zoletil-xylazine anesthesia [14, 15]. In animals of groups 2 and 3, skin incision with the length of up to 2 cm was performed between the left auricle and left eye. In the incision site, soft tissues were dissected up to the skull bones, and a burr hole 5 mm in diameter was created using a high-speed bur (20,000 rpm) with constant irrigation-induced cooling. Selective diathermocoagulation of the cerebral pial vessels (15 V, 3 s) in the cortical zone of the middle cerebral artery was performed using the operating microscope with the 10× magnification. All methodological aspects of the study were compliant with modern standards of experimental cerebral ischemia modeling [16, 17]. Animals of group 1 underwent all consecutive surgical interventions, including selective diathermocoagulation of the cerebral pial vessels.

Three animals were excluded from further assessment (two in group 2 and one in group 3), since these died within 6 h after surgery. Thus, three groups of animals were created: group 1 (n = 10), group 2 (n = 8), group 3 (n = 9).

On days 3 and 7, neurological status was assessed in all groups using the Garcia JH score, Placing test, Bederson test [18–20]. The results of each particular test were expressed in points.

Microcirculation rate (MR) was assessed in ischemic brain injury focus by laser Doppler flowmetry for 5 min using the LAAK-01 system (Lazma, Russia). The values were processed using the software package by Lazma (Russia), and MR was calculated using the following formula: MR = Ne + Vav, where Ne was the concentration of erythrocytes in the probed tissue volume, Vav was the average erythrocyte sedimentation rate. MR was expressed in perfusion units (PU).

Statistical data processing was performed using the IBM SPSS Statistics 19 software package. The quantitative data distribution was tested for normality using the Shapiro–Wilk test. Since the distribution of most studied parameters was non-normal, nonparametric methods were used for analysis. Intergroup comparison was performed using the Kruskal–Wallis test (when comparing three or more groups), Mann–Whitney U-test (when performing pairwise comparison of groups). The paired Wilcoxon signed-rank test for related samples was used to estimate the intragroup dynamics (to compare the values on days 3 and 7); Spearman's rank correlation coefficient (r) was used for correlation analysis. The data were presented as the median (Me), lower and upper quartiles (Q_1; Q_3). The differences were considered significant at $p < 0.05$.

RESULTS

Animals with EACI showed focal neurological deficit in the form of right hemiparesis, stato-locomotor disorder on days 3 and 7. Animals with EACI demonstrated a significant decrease in Garcia JH scores by 58.3% (p = 0.001), Placing test scores by 57.9% (p = 0.002), along with the significant increase in Bederson test scores compared to SO animals; on day 7, the significant decrease in Garcia JH scores by 75% ($p < 0.001$), Placing test scores by 78.9% ($p < 0.001$), along with the increase in Bederson test scores were reported. Animals with EACI showed the significant decrease in Garcia JH scores by 40% (p = 0.008), Placing test scores by 50.0% (p = 0.003), along with the increase in Bederson test scores by 100% (p = 0.001) on day 7 compared to day 3 (Table 1).

Microcirculation assessment showed the decrease in MR on day 3 by 30% (p = 0.02), by 38% on day 7 (p = 0.005) in animals with EACI compared to SO ones. Animals with EACI showed the significant MR decrease by 11% (p = 0.03) on day 7 compared to day 3 (Table 2).

Thus, focal neurological deficit and the decrease in cerebral blood flow in the ischemic brain lesion were reported in animals with EACI on days 3 and 7, which was confirmed by the significant decrease in Garcia JH, Placing test scores, as well as by the increase in Bederson test scores and MR decrease.

Table 1. Effect of MT on neurological status in EACI (Me [Q_1; Q_3])

Indicator	Group 1		Group 2		Group 3	
	Day 3 (n = 10)	Day 7 (n = 10)	Day 3 (n = 8)	Day 7 (n = 8)	Day 3 (n = 9)	Day 7 (n = 9)
Garcia JH score	18.00 [18.00; 18.00]	18.00 [18.00; 18.00]	7.50 [7.00; 8.00]*	4.50 [3.00; 6.00] *&	11.50 [10.00; 13.00] *#	15.00 [14.00; 16.00] *#&
Placing test score	9.50 [9.00; 10.00]	9.50 [9.00; 10.00]	2.00 [2.00; 3.00]*	4.00 [4.00; 4.00] *&	1.00 [1.00; 2.00] *#	0.00 [0.00; 1.00] *#&
Bederson test score	0.00 [0.00; 0.00]	0.00 [0.00; 0.00]	4.00 [3.00; 4.00]*	2.00 [1.00; 2.00] *&	6.00 [6.00; 7.00] *#	8.50 [8.00; 9.00] *#&

Note: significant differences based on the Mann–Whitney *U*-test are designated as follows: * — compared to group 1 ($p < 0.05$); # — compared to group 2 ($p < 0.05$); & — significant differences based on the Wilcoxon test between days 3 and 7 within the same group ($p < 0.05$).

The use of MT-based rectal suppositories on days 3 and 7 led to the right hemiparesis and stato-locomotor disorder severity decrease. The animals receiving MT showed the significant increase in the Garcia JH scores by 53.3% ($p = 0.008$), Placing test scores by 50% ($p = 0.016$), along with the significant decrease in Bederson test scores by 50% ($p = 0.029$) compared to animals with EACI on day 3; on day 7, the significant increase in Garcia JH scores by 233% ($p < 0.0001$), Placing test scores by 325% ($p < 0.0001$) and the significant decrease in Bederson test scores by 100% ($p < 0.0001$) were reported. The significant increase in Garcia JH scores by 30.4% ($p = 0.004$), Placing test scores by 41.7% ($p = 0.002$) and the significant decrease in Bederson test scores by 100% ($p = 0.003$) upon MT administration on day 7 compared to day 3 were reported (Table 1).

Microcirculation assessment showed the increase in MR by 12.5% on day 3 ($p = 0.016$), by 43.9% on day 7 ($p = 0.005$) in the animals receiving MT compared to the ones with EACI. The animals showed the significant MR increase by 13.8% ($p = 0.028$) upon MT administration on day 7 compared to day 3 (Table 2).

The animals receiving MT showed the significant decrease in Garcia JH scores by 36.1% ($p < 0.001$), Placing test scores by 36.8% ($p < 0.001$) and the significant increase in Bederson test scores by 100% ($p = 0.002$) compared to SO animals on day 3; on day 7, these demonstrated the decrease in Garcia JH scores by 16.7% ($p < 0.008$), Placing test scores by 10.5% ($p < 0.038$) and the significant increase in Bederson test scores by 0% ($p = 0.157$) (Table 1).

The correlation analysis of Garcia JH scores, Placing test scores, and MR values on days 3 and 7 revealed a positive correlation, along with the negative correlation between Bederson test scores and MR: the neurological deficit improvement in animals with EACI in the context of using MT-based rectal suppositories was associated with the MR increase in the ischemic brain lesion (Table 3).

DISCUSSION

It can be assumed that neurological deficit we have detected results from local critical decrease in cerebral blood flow in the brain matter and activation of the cascade of pathochemical reactions, including mitochondrial dysfunction with subsequent neuronal energy deficiency, oxidative stress activation, glutamate-induced excitotoxicity, neuroinflammation resulting in neuronal damage in the ischemic injury focus [21]. The use of MT-based rectal suppositories resulted in partial restoration of neurological status and cerebral blood flow in the ischemic injury focus, which could be due to the MT pharmacological features and pleiotropic effects, including neuroprotective and vasoactive effects. The MT relatively low molecular weight (232 g/mol), moderate lipid solubility (Log $p = 3$), and moderate bioavailability when administered rectally (54–72%) can ensure penetration of the blood-brain barrier and have a systemic effect on the ischemic injury focus and potential penetration [22, 23]. We believe that MT contained in the rectal suppositories entered the systemic blood flow and reached the ischemic lesion in the brain, showing the neuroprotective effect. The MT pleiotropic effects determining its possible neuroprotective effect can be associated with the activity mediated by specific membrane and nuclear receptors (MT1, MT2, ROR), as well as with the direct effect [24]. The MT binding to the MT receptors of microglial cells through the STAT3 pathway inhibition contributed to the reduced synthesis of pro-inflammatory cytokines in the ischemic injury focus and reduced neuroinflammation [25]. Furthermore, MT could cause inhibition of NADPH reductase activity and enhanced synthesis of glutathione peroxidase, thereby decreasing the activity of oxidative stress processes in the ischemic brain injury focus [26]. MT enhanced the 90RSK activity resulting in the Bad pro-apoptotic protein inactivation through ERK1 phosphorylation, thereby contributing to the increased survival of neurons in the ischemic injury zone [27]. The MR increase in the ischemic brain injury focus can be associated with the vasoactive effect of MT, which enhanced NO synthesis in endothelial cells and increased cerebral blood flow through the increased activity of endothelial NO synthase [28]. The findings clearly demonstrate clinical prospects for the use of MT-based rectal suppositories as adjunctive therapy for IS, especially in the elderly and seriously ill patients, in whom standard treatment methods show limited efficacy. The data obtained provide a strong basis for further research aimed at in-depth study of the mechanisms underlying the MT neuroprotective effect via monitoring of the dynamic changes in the levels of biochemical markers (MDA, S100β) and their correlation with clinical outcomes. The promising areas for scientific research can be

Table 2. Effect of MT on microcirculation values in EACI (Me [Q_1; Q_3])

Indicator	Group 1 (n = 10)		Group 2		Group 3	
	Day 3 (n = 10)	Day 7 (n = 10)	Day 3 (n = 10)	Day 7 (n = 10)	Day 3 (n = 10)	Day 7 (n = 10)
Microcirculation value, PU	22.11 [20.65; 22.69]	22.11 [20.65; 22.69]	15.51 [15.48; 15.88]*	13.79 [13.49; 14.09]*&	17.45 [17.33; 18.83]*#	19.85 [19.44; 19.90]*#&

Note: significant differences based on the Mann–Whitney U-test are designated as follows: * — compared to group 1 ($p < 0.05$); # — compared to group 2 ($p < 0.05$); & — significant differences based on the Wilcoxon test between days 3 and 7 within the same group ($p < 0.05$).

Table 3. Correlation between neurological status indicators and MR in EACI in the context of using MT-based rectal suppositories

Indicators	Microcirculation rate, PU	
	Day 3	Day 7
Garcia JH score	$r = 0.63$	$r = 0.67$
Placing Test score	$r = 0.77$	$r = 0.64$
Bederson Test score	$r = -0.61$	$r = -0.60$

Note: r — Spearman's rank correlation coefficient ($r < 0.05$).

as follows: development of differentiated MT dosing algorithms considering the patients' individual characteristics; assessment of delayed therapy effects between 6 and 12 months of follow-up; study of the possibilities of using combination therapy with other neuroprotective agents. Successful introduction of this method into wide clinical practice requires large-scale multicenter randomized trials involving the use of standardized assessment protocols. The development of personalized approaches to therapy considering the age-related specifics, comorbidities, and individual characteristics of the patients' cerebral hemodynamics is of special importance.

CONCLUSIONS

Selective diathermocoagulation of the cerebral pial vessels performed on days 3 and 7 resulted in the critical cerebral blood flow decrease with subsequent development of neurological deficit in the form of right hemiparesis and stato-locomotor disorder manifested by the progressive decrease in Garcia JH, Placing test scores and increase in Bederson test scores, as well as in the reduced MR in the ischemic brain injury focus. The use of original rectal suppositories with the weight of 100 mg containing 2.5 mg of MT every 24 h throughout 7 days resulted in partial neurological deficit restoration manifested by the increase in Garcia JH, Placing test scores, decrease in Bederson test scores and increased MR not reaching the values of the SO animals in the ischemic brain injury focus. The correlation analysis revealed the association between the neurological status indicators and MR: the neurological deficit improvement in animals with EACI in the context of using MT-based rectal suppositories was associated with the MR increase in the ischemic brain injury focus.

References

1. Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, et al. World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. Int J Stroke. 2022; 17 (1): 18–29.
2. Ignateva VI, Voznjuk IA, Shamalov NA, Reznik AV, Vinickij AA, Derkach EV. Social'no-jekonomicheskoe bremja insul'ta v Rossijskoj Federacii. Zhurnal nevrologii i psihiatrii im. S. S. Korsakova. Specvypuski. 2023; 123 (8–2): 5–15. Russian.
3. Cao Y, Yue X, Jia M, Wang J. Neuroinflammation and anti-inflammatory therapy for ischemic stroke. Heliyon. 2023; 9 (7): e17986.
4. Paul S, Candelario-Jalil E. Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies. Exp Neurol. 2021; 335: 113518.
5. Ma N, Zhang J, Reiter R J, Ma X. Melatonin mediates mucosal immune cells, microbial metabolism, and rhythm crosstalk: A therapeutic target to reduce intestinal inflammation Med Res Rev. 2020; 40 (2): 606–32.
6. Gancgorn EV, Hloponin DP, Makljakov YuS. Patofiziologicheskie osnovy sovremennoj farmakoterapii ostroj ishemii golovnogo mozga. Mesto nootropov i antioksidantov v nejroprotekcii. Medicinskij vestnik Juga Rossii. 2013; (2): 4–12. Russian.
7. Yang Y, Jiang S, Dong Y, Fan C, Zhao L, Yang X, Li J, et al. Melatonin prevents cell death and mitochondrial dysfunction via a SIRT1-dependent mechanism during ischemic-stroke in mice. J Pineal Res. 2015; 58 (1): 61–70.
8. Pallab B, Kumar PA, Sudip P, Ranjana P. Melatonin renders neuroprotection by Protein Kinase C mediated Aquaporin-4 inhibition in animal model of focal cerebral ischemia. Life Sciences. 2014; 100 (2): 97–109.
9. Ma Q, Reiter RJ, Chen Y. Role of melatonin in controlling angiogenesis under physiological and pathological conditions. Angiogenesis. 2020; 23 (2): 91–104.
10. Qin T, Feng D, Zhou B, Bai L, Yin Y. Melatonin Suppresses LPS-Induced Oxidative Stress in Dendritic Cells for Inflammatory Regulation via the Nrf2/HO-1 Axis. Antioxidants (Basel). 2022; 11 (10): 2012.
11. Khedr EM, Abbass MA, Soliman RK, Radwa KS, Ahmed FZ, Gamea A. Post-stroke dysphagia: frequency, risk factors, and topographic representation: hospital-based study. Egypt J Neurol Psychiatry Neurosurg. 2021; 57 (23).
12. Brinkwirth S, Ayobami O, Eckmanns T, Markwart R. Hospital-acquired infections caused by enterococci: a systematic review and meta-analysis, WHO European Region, 1 January 2010 to 4 February 2020. Euro Surveill. 2021; 26(45):2001628.
13. Osikov MV, Ushakova VA, Grekova IV, Bojko MS, Grechishkin MV, avtory; FGBOU VO «Juzhno-Ural'skij gosudarstvennyj medicinskij universitet Minzdrava Rossii», patentoobladatel'. Sredstvo s melatoninom dlja terapii vospalitel'nyh zabolevanij kishechnika v forme rektal'nyh suppozitoriev. Patent RF № 2819721 07.06.2024. Russian.
14. Chen ST, Hsu CY, Hogan EL, Maricq H, Balentine JD. A model of focal ischemic stroke in the rat: reproducible extensive cortical infarction. Stroke. 1986; 17 (4): 738–43.
15. Kadomcev DV, Pasechnikova EA, Golubev VG. Zoletil-ksilazinovyj narkoz v jeksperimentah u krys. Mezhdunarodnyj zhurnal prikladnyh i fundamental'nyh issledovanij. 2015; 5: 56–57. Russian.
16. Mirzojan RS, Plotnikov MB, Gan'shina TS, i dr. Metodicheskie rekomendacii po doklinicheskomu izucheniju lekarstvennyh sredstv dlja lechenija narushenij mozgovogo krovoobrashhenija i migreni. Rukovodstvo po provedeniju doklinicheskih issledovanij lekarstvennyh sredstv. Chast' pervaja. M.: Grif i K, 2012: 480–7. Russian.
17. Habriev RU, redaktor. Rukovodstvo po jeksperimental'nomu (doklinicheskomu) izucheniju novyh farmakologicheskih veshhestv. M.: Medicina, 2005; 832 s. Russian.
18. Schallert MT, Whishaw IQ. The Behavior of the Laboratory Rat: A Handbook with Tests Oxford University Press. 2004: 129–40.
19. Schaar KL, Brenneman MM, Savitz SI. Functional assessments in the rodent stroke model. Exp Transl Stroke Med. 2010; 2 (1): 13.
20. Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke. 1986; 17 (3): 472–6.
21. Paul S, Candelario-Jalil E. Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies. Exp Neurol. 2021; 335: 113518.

22. Skinner DC, Malpaux B. High melatonin concentrations in third ventricular cerebrospinal fluid are not due to Galen vein blood recirculating through the choroid plexus. Endocrinology. 1999; 140 (10): 4399–405.
23. Tricoire H, et al. Melatonin enters the cerebrospinal fluid through the pineal recess. Endocrinology. 2003; 144 (1): 84–90.
24. Liu L, Labani N, Cecon E, Jockers R. Melatonin Target Proteins: Too Many or Not Enough? Front Endocrinol (Lausanne). 2019; 10: 791.
25. Liu ZJ, Ran YY, Qie SY, Gong WJ, Gao FH, Ding ZT, et al. Melatonin protects against ischemic stroke by modulating microglia/macrophage polarization toward anti-inflammatory phenotype through STAT3 pathway. CNS Neurosci Ther. 2019; 25 (12): 1353–62.
26. Wang J, Gao S, Lenahan C, Gu Y, Wang X, Fang Y, et al. Melatonin as an Antioxidant Agent in Stroke: An Updated Review. Aging Dis. 2022; 13 (6): 1823–44.
27. Zhang C, Ma Y, Zhao Y, Guo N, Han C, Wu Q, et al. Systematic review of melatonin in cerebral ischemia-reperfusion injury: critical role and therapeutic opportunities. Front Pharmacol. 2024; 15: 1356112.
28. Aladag MA, Turkoz Y, Parlakpinar H, Ozen H, Egri M, Unal SC. Melatonin ameliorates cerebral vasospasm after experimental subarachnoidal haemorrhage correcting imbalance of nitric oxide levels in rats. Neurochem Res. 2009; 34 (11): 1935–44. DOI: 10.1007/s11064-009-9979-7. Epub 2009 May 5. PMID: 19415488.

Литература

1. Feigin VL, Brainin M, Norrving B, Martins S, Sacco RL, Hacke W, et al. World Stroke Organization (WSO): Global Stroke Fact Sheet 2022. Int J Stroke. 2022; 17 (1): 18–29.
2. Игнатьева В. И., Вознюк И. А., Шамалов Н. А., Резник А. В., Виницкий А. А., Деркач Е. В. Социально-экономическое бремя инсульта в Российской Федерации. Журнал неврологии и психиатрии им. С. С. Корсакова. Спецвыпуски. 2023; 123 (8–2): 5–15.
3. Cao Y, Yue X, Jia M, Wang J. Neuroinflammation and anti-inflammatory therapy for ischemic stroke. Heliyon. 2023; 9 (7): e17986.
4. Paul S, Candelario-Jalil E. Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies. Exp Neurol. 2021; 335: 113518.
5. Ma N, Zhang J, Reiter R J, Ma X. Melatonin mediates mucosal immune cells, microbial metabolism, and rhythm crosstalk: A therapeutic target to reduce intestinal inflammation Med Res Rev. 2020; 40 (2): 606–32.
6. Ганцгорн Е. В., Хлопонин Д. П., Макляков Ю. С. Патофизиологические основы современной фармакотерапии острой ишемии головного мозга. Место ноотропов и антиоксидантов в нейропротекции. Медицинский вестник Юга России. 2013; (2): 4–12.
7. Yang Y, Jiang S, Dong Y, Fan C, Zhao L, Yang X, Li J, et al. Melatonin prevents cell death and mitochondrial dysfunction via a SIRT1-dependent mechanism during ischemic-stroke in mice. J Pineal Res. 2015; 58 (1): 61–70.
8. Pallab B, Kumar PA, Sudip P, Ranjana P. Melatonin renders neuroprotection by Protein Kinase C mediated Aquaporin-4 inhibition in animal model of focal cerebral ischemia. Life Sciences. 2014; 100 (2): 97–109.
9. Ma Q, Reiter RJ, Chen Y. Role of melatonin in controlling angiogenesis under physiological and pathological conditions. Angiogenesis. 2020; 23 (2): 91–104.
10. Qin T, Feng D, Zhou B, Bai L, Yin Y. Melatonin Suppresses LPS-Induced Oxidative Stress in Dendritic Cells for Inflammatory Regulation via the Nrf2/HO-1 Axis. Antioxidants (Basel). 2022; 11 (10): 2012.
11. Khedr EM, Abbass MA, Soliman RK, Radwa KS, Ahmed FZ, Gamea A. Post-stroke dysphagia: frequency, risk factors, and topographic representation: hospital-based study. Egypt J Neurol Psychiatry Neurosurg. 2021; 57 (23).
12. Brinkwirth S, Ayobami O, Eckmanns T, Markwart R. Hospital-acquired infections caused by enterococci: a systematic review and meta-analysis, WHO European Region, 1 January 2010 to 4 February 2020. Euro Surveill. 2021; 26(45):2001628.
13. Осиков М. В., Ушакова В. А., Грекова И. В., Бойко М. С., Гречишкин М. В., авторы; ФГБОУ ВО «Южно-Уральский государственный медицинский университет Минздрава России», патентообладатель. Средство с мелатонином для терапии воспалительных заболеваний кишечника в форме ректальных суппозиториев. Патент РФ № 2819721 07.06.2024.
14. Chen ST, Hsu CY, Hogan EL, Maricq H, Balentine JD. A model of focal ischemic stroke in the rat: reproducible extensive cortical infarction. Stroke. 1986; 17 (4): 738–43.
15. Кадомцев Д. В., Пасечникова Е. А., Голубев В. Г. Золетил-ксилазиновый наркоз в экспериментах у крыс. Международный журнал прикладных и фундаментальных исследований. 2015; 5: 56–57.
16. Мирзоян Р. С., Плотников М. Б., Ганьшина Т. С. и др. Методические рекомендации по доклиническому изучению лекарственных средств для лечения нарушений мозгового кровообращения и мигрени. Руководство по проведению доклинических исследований лекарственных средств. Часть первая. М.: Гриф и К, 2012: 480–7.
17. Хабриев Р. У., редактор. Руководство по экспериментальному (доклиническому) изучению новых фармакологических веществ. М.: Медицина, 2005; 832 с.
18. Schallert MT, Whishaw IQ. The Behavior of the Laboratory Rat: A Handbook with Tests Oxford University Press. 2004: 129–40.
19. Schaar KL, Brenneman MM, Savitz SI. Functional assessments in the rodent stroke model. Exp Transl Stroke Med. 2010; 2 (1): 13.
20. Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. Stroke. 1986; 17 (3): 472–6.
21. Paul S, Candelario-Jalil E. Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies. Exp Neurol. 2021; 335: 113518.
22. Skinner DC, Malpaux B. High melatonin concentrations in third ventricular cerebrospinal fluid are not due to Galen vein blood recirculating through the choroid plexus. Endocrinology. 1999; 140 (10): 4399–405.
23. Tricoire H, et al. Melatonin enters the cerebrospinal fluid through the pineal recess. Endocrinology. 2003; 144 (1): 84–90.
24. Liu L, Labani N, Cecon E, Jockers R. Melatonin Target Proteins: Too Many or Not Enough? Front Endocrinol (Lausanne). 2019; 10: 791.
25. Liu ZJ, Ran YY, Qie SY, Gong WJ, Gao FH, Ding ZT, et al. Melatonin protects against ischemic stroke by modulating microglia/macrophage polarization toward anti-inflammatory phenotype through STAT3 pathway. CNS Neurosci Ther. 2019; 25 (12): 1353–62.
26. Wang J, Gao S, Lenahan C, Gu Y, Wang X, Fang Y, et al. Melatonin as an Antioxidant Agent in Stroke: An Updated Review. Aging Dis. 2022; 13 (6): 1823–44.
27. Zhang C, Ma Y, Zhao Y, Guo N, Han C, Wu Q, et al. Systematic review of melatonin in cerebral ischemia-reperfusion injury: critical role and therapeutic opportunities. Front Pharmacol. 2024; 15: 1356112.
28. Aladag MA, Turkoz Y, Parlakpinar H, Ozen H, Egri M, Unal SC. Melatonin ameliorates cerebral vasospasm after experimental subarachnoidal haemorrhage correcting imbalance of nitric oxide levels in rats. Neurochem Res. 2009; 34 (11): 1935–44. DOI: 10.1007/s11064-009-9979-7. Epub 2009 May 5. PMID: 19415488.

COMPARISON OF THE EFFICACY OF MRNA VACCINES AGAINST *M. TUBERCULOSIS* BASED ON LINEAR AND CIRCULAR RNAS

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The success of mRNA-based vaccine formulations against viral infections motivated many researchers to develop mRNA vaccines against bacterial infections. The development of new anti-tuberculosis vaccine is an urgent task since the only approved BCG vaccine is not effective enough in terms of infection prevention, despite the fact that it reduces the risk of severe disease. The study aimed to compare two anti-tuberculosis mRNA vaccines based on the classic linear mRNA (mRNA-MTB-mEp-5-1) and circular RNA (circRNA-MTB-mEp-5-1) by immunogenicity and the capability of protecting I/St mice against *M. tuberculosis* infection. The efficacy of mRNA vaccines in the formulations with lipid nanoparticles was compared with the BCG efficacy. The findings suggest that immunization with the mRNA vaccine based on the linear mRNA resulted in the cell-based and humoral immune response (OD IgG = 0.36 ± 0.12) that was less pronounced than after BCG vaccination (OD IgG = 0.54 ± 0.14). At the same time, immunization with the mRNA vaccine and BCG ensured comparable reduction of bacterial load in the lung and spleen of experimental mice (CFU in lung tissue for BCG: $4.00 \times 10^5 \pm 2.13 \times 10^5$, $p = 0.0068$; mRNA: $4.72 \times 10^5 \pm 3.44 \times 10^5$, $p = 0.0059$; LNP: $4.91 \times 10^6 \pm 3.89 \times 10^6$, ns; PBS: $4.01 \times 10^6 \pm 1.69 \times 10^6$) and increased survival of mice after getting infected with *M. tuberculosis*. Immunization with the vaccine based on the circular RNA resulted in developing humoral mmunity only (OD IgG = 0.52 ± 0.13) and did not ensure protection after getting infected with *M. tuberculosis* (CFU in the lung for circRNA: $2.12 \times 10^6 \pm 5.30 \times 10^5$, $p = 0.85$). Thus, in our studies, anti-tuberculosis vaccines based on circular RNAs are inferior in effectiveness to formulations based on linear RNAs.

Keywords: mRNA vaccine, circular RNA, tuberculosis, immunogenicity, protective immunity

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Compliance with ethical standards: the study was approved by the Ethics Committee of the Central Tuberculosis Research Institute (protocol No. 3/2 dated 11 May 2022) and conducted in accordance with the Order of the Ministry of Health No. 755 and the Guidelines issued by the Office of Laboratory Animal Welfare (A5502-01).

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СРАВНЕНИЕ ЭФФЕКТИВНОСТИ МРНК ВАКЦИН ПРОТИВ *M. TUBERCULOSIS* НА ОСНОВЕ ЛИНЕЙНЫХ И КОЛЬЦЕВЫХ РНК

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Успехи вакцинных препаратов на основе мРНК против вирусных инфекций побудили многих исследователей к разработке мРНК-вакцин против бактериальных инфекций. Разработка новой вакцины против туберкулеза является актуальной задачей, поскольку единственная одобренная к использованию вакцина BCG, хотя и снижает риск развития тяжелых форм заболевания, не является достаточно эффективной для предотвращения инфицирования. Целью исследования было сравнить две мРНК-вакцины против туберкулеза на основе классической линейной мРНК (mRNA-MTB-mEp-5-1) и на основе кольцевой мРНК (circRNA-MTB-mEp-5-1) по иммуногенности и способности защищать мышей I/St от заражения *M. tuberculosis*. Эффективность мРНК-вакцин в составе с липидными наночастицами сравнивали с эффективностью BCG. Результаты свидетельствуют о том, что иммунизация мРНК-вакциной на основе линейной мРНК привела к формированию клеточного и гуморального иммунного ответа (OD IgG = 0,36 ± 0,12), который был менее выражен, чем после вакцинации BCG (OD IgG = 0,54 ± 0,14). В то же время иммунизация мРНК-вакциной и BCG обеспечила сопоставимое снижение бактериальной нагрузки в легких и селезенке экспериментальных мышей (КОЕ в легочной ткани BCG: $4,00 \times 10^5 \pm 2,13 \times 10^5$, $p = 0,0068$; линРНК: $4,72 \times 10^5 \pm 3,44 \times 10^5$, $p = 0,0059$; LNP: $4,91 \times 10^6 \pm 3,89 \times 10^6$, ns; PBS: $4,01 \times 10^6 \pm 1,69 \times 10^6$) и повысила их выживаемость после заражения *M. tuberculosis*. Иммунизация вакциной на основе кольцевой мРНК привела к формированию только гуморального иммунитета (OD IgG = 0,52 ± 0,13) и не обеспечила защиту после заражения *M. tuberculosis* (КОЕ в легких колРНК: $2,12 \times 10^6 \pm 5,30 \times 10^5$, $p = 0,85$). Таким образом, в наших исследованиях противотуберкулезные вакцины на основе кольцевых мРНК уступают в эффективности препаратам на основе линейных РНК.

Ключевые слова: мРНК-вакцина, кольцевые мРНК, туберкулез, иммуногенность, протективный иммунитет

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Tuberculosis (TB) is a highly contagious disease caused by the bacterium *Mycobacterium tuberculosis*, which is still the leading cause of death from infectious diseases. In 2023, a total of 10.8 million of incident TB cases and 1.25 million of deaths from this infection were reported [1]. Tuberculosis prevention is hampered by often asymptomatic course of the early-stage disease and delayed manifestation, which make it difficult to monitor the epidemiological situation. The spread of antimicrobial-resistant mycobacteria is associated with further challenges. TB is still a deadly disease, and the search for effective prevention and treatment methods is an urgent problem.

The development and introduction of a new tuberculosis vaccine represents the most important step on the way to elimination of this dangerous infection. BCG vaccine is not recommended by the World Health Organization for tuberculosis prevention in adults due to the risk of adverse effects. Variable efficacy in different geographic regions, strain heterogeneity, and low efficacy against pulmonary tuberculosis being the most prevalent tuberculosis form can be also considered the BCG disadvantages [2]. Thus, there is an urgent need to create new drugs capable of preventing TB in all age groups. Currently, a total of 15 vaccines are through various phases of clinical development: between phase I and phase III clinical trials [3]. All these represent different formulation types: viral vector-based, inactivated mycobacteria, live-attenuated mycobacteria, protein subunit and mRNA vaccines.

mRNA vaccines have become widely used due to the success of the RNA-1273 (Moderna) and BNT162b2 (Pfizer) vaccines against SARS-CoV2 [4]. This approach to vaccination has a number of benefits compared to other immunization variants: low cost of production, rapid development, high efficacy, lack of infectivity, and no integration into the genome. At the same time, the experience of using mRNA vaccines against infectious diseases caused by bacteria is limited [5]. Moreover, not all mRNA-based formulations show high efficacy: low RNA stability in the cell results in premature RNA degradation, reduced target protein translation [6]. The use of the alternative mRNA platforms, such as self-amplifying and circular RNAs, are considered as a possible way to solve these problems.

Circular RNAs were discovered in the 1980s. Since then these attracted attention due to their features. In contrast to linear mRNAs, the circular RNA-based formulations are less prone to degradation due to the lack of free 5' or 3' end, through which cleavage by exonucleases occurs [7]. Furthermore, circular RNAs are not subject to the degradation mechanisms, such as nonsense-mediated mRNA decay (NMD) or non-stop decay (NSD) [8]. Circular RNAs are translated through IRES (internal ribosome entry site), the indirect mechanism activated primarily under conditions of cellular stress, when the cap-dependent translation is inhibited; it is ensured by recruiting of the so-called IRES-transacting factors (ITAF) together with the eIF and eEF [9]. Currently, no clinical trials of the circular RNA-based tuberculosis vaccines are conducted [3]. At the same time, the circular RNA-based formulations have shown high efficacy in experimental models of viral infections [10, 11]; these are currently tested in clinical trials of the drugs for radiation-induced xerostomia [NCT06714253] and SARS-CoV-2 [NCT06205524]. We assume that these features of circular RNAs can make it possible to consider the circular RNA-based formulations as a promising platform for future tuberculosis vaccines. Therefore, the aim of our study was to evaluate the effectiveness of an anti-TB vaccine based on circular RNA.

METHODS

Animals

The experiments involved female inbred C57BL/6Cit (B6) and I/StSnEgYCit (I/St) mice obtained from the breeding nursery of the Central Tuberculosis Research Institute. The animals aged 2–4 months with the body weight of 20–25 g were included in the study. Lineages were maintained through brother-sister inbreeding; the animals had *ad libitum* access to food and water. A total of 25 B6 and 75 I/St females were used.

Experimental design

A total of 5 experimental groups for each mouse lineage were formed for the study: for B6 (5 animals per group) and I/St (15 animals per group) mice.

1. Group with the double intramuscular administration of the mRNA-MTB-mEp5-1 vaccine in a dose of 5 μg/mouse with the 4-week interval between injections.
2. Group with the double intramuscular administration of the circRNA-MTB-mEp5-1 vaccine in a dose of 5 μg/animal with the 4-week interval between injections.
3. Group with a single BCG administration in a dose of 1×10^5 CFU/mouse (5 weeks before tissue collection).
4. Control group with the double intramuscular administration of the lipid nanoparticles (LNP) with no mRNA in a dose equivalent to that administered to groups I–II with the 4-week interval between injections.
5. Control group with the double intramuscular administration of phosphate buffered saline (PBS) with the 4-week interval between injections.

The B6 mice were used to assess T cell-mediated response (ELISpot), as well as to assess the titer of IgG against antigens of *M. tuberculosis*.

The I/St mice were used to assess protective immune response, since mice of this lineage show increased sensitivity to tuberculosis infection [12]. The I/St mice were intravenously infected with the virulent *M. tuberculosis* strain 4 weeks after the second vaccination with mRNA vaccines / 5 weeks after BCG vaccination. Mycobacterial load in the spleen and lung (5 mice in each group) and the dynamics of deaths of animals after getting infected (10 mice in each group) were assessed 50 days after infection in these mice.

Obtaining mRNA vaccines

The MTB-mEp5-1 multi-epitope mRNA vaccine was described in detail earlier [13]. The coding sequence for the circMTB-mEp5-1 circular RNA-based vaccine was the same as that for MTB-mEp5-1. The cassette for circRNA comprised type I introns from Anabaena (on the 5' and 3' ends), homology arms, spacers, type I IRES (CVB3). The construct was also built by PCR and cloned into pSmart (Lucigen, USA) at the *EcoRI* and *AhlI* restriction endonuclease sites. The MTB-mEp5-1 circular RNA sequence is provided in Appendix.

RNA synthesis was accomplished using the mRNA-20 kit (Biolabmix, Russia) supplemented with pyrophosphatase (NEB, USA) and RNAse inhibitor (Biolabmix, Russia). The $m_2^{7,3'}$-OGpppAmG synthetic cap analogue (Biolabmix, Russia) was used for *in vitro* linear RNA transcription. To obtain circular RNA, guanosine triphosphate (GTP) was added to the reaction mixture after the transcription termination to a final concentration of 2 mM (Biolabmix, Russia) in order to induce circularization. The mixture was incubated at 55 °C for 15 min,

cooled on ice for 5 min and purified on the magnetic particles (VAHTS RNA Clean Beads, China). To eliminate linear RNA forms from the circular RNA-based formulation, the sample was treated with RNAse R (Abcam, USA). For that RNA was heated at 70 °C for 3 min, then cooled rapidly on ice (5 min). After that we added 0.5 µL of RNAse R (Abcam, USA) per 20 µg of RNA and 10× RNAse R buffer and incubated at 37 °C for 7 min, after the end of which another 0.5 µL of RNAse R were added, further incubated for 7 min at 37 °C and purified on the magnetic particles (VAHTS RNA Clean Beads, China).

After the *in vitro* transcription termination, the DNA matrix was eliminated by treating with 2 U of DNAse I (NEB, USA) per 1 µg of DNA at the temperature of 37 °C for 15 min. RNA was purified using magnetic particles (VAHTS RNA Clean Beads, China) in accordance with the manufacturer's instructions. The RNA quality was assessed by capillary electrophoresis (Qsep1-Plus, BiOptic, Taiwan).

mRNA was formulated into lipid nanoparticles using the microfluid cartridge in the NanoAssemblr™ Benchtop system, as earlier reported [14]. RNA was mixed with the lipid mixture ethanol solution in the microfluid cartridge; the aqueous and ethanol phases were mixed in a ratio of 3 : 1 v/v. The five-component lipid mixture consisted of the ionized lipidoid ALC-0315 (Sinopeg, Xiamen, China), SM-102 (Sinopeg, Xiamen, China), DSPC (Avanti Polar Lipids, USA), cholesterol (Merck Millipore, USA), and DMG PEG-2000 (Merck Millipor, USA) in a molar ratio (%) of 23.15 : 23.15 : 9.4 : 42.7 : 1.6. Particles were concentrated and sterilized using the PES membrane with the pore diameter of 0.22 µm. The quality of the LNPs obtained was assessed based on three parameters: particle size, polydispersity index (PDI) (Zetasizer Ultra ZSP; Malvern PanalityCal, USA), and mRNA payload of the particles. The particle size for the MTB-mEp-5-1 sample was 90.5 nm, polydispersity index was 0.22. The hydrodynamic diameter for the circMTB-mEp-5-1 formulation was 85.7 nm, polydispersity index was 0.19. The RNA payload was above 90% in both formulations. Lipid nanoparticles were stored at +4 °C for no more than 2 weeks prior to formulation administration.

T-cell response assessment: quantification of IFNγ-producting cells

The level of protective T-cell immune response in B6 mice was assessed based on the counts of cells secreting IFNγ in response to stimulation with mycobacterial antigens isolated from the spleen by the ELISpot method using the Mouse IFNγ ELISpot Set (BD; USA) and AEC Substrate Set (BD; USA) kits in accordance with the manufacturers' instructions [15]. The M. tuberculosis sonicate in a dose of 10 µg/mL was used as a source of mycobacterial antigens. Sonicate for the study was kindly provided by V.G. Avdienko. To produce it, mycobacteria were grown in the Sauton medium for 28 days at 37 °C. Sonicate was obtained from the washed clean bacterial mass using the MSE ultrasonic disintegrator by the method earlier reported by V.G. Avdienko et al. [16].

Humoral immunity assessment: determination of the titer of IgG immunoglobulins against *M. tuberculosis* sonicate

The titer of the IgG immunoglobulin against the *M. tuberculosis* sonicate was determined in the serum samples of experimental animals. Blood serum of the B6 experimental mice was subjected to titration in the phosphate buffered saline (PBS) from 1 : 50 to 1 : 400. The humoral immune response intensity was assessed based on optical density (OD) by the earlier reported method [17].

Vaccination protective effect assessment

To induce experimental tuberculosis infection 4 weeks after the second mRNA vaccine dose, the I/St mice were intravenously infected by the virulent *M. tuberculosis* H37Rv strain (Pasteur) from the collection of the Central Tuberculosis Research Institute in a dose of 5×10^5 CFU per mouse. BCG vaccination was performed 5 weeks before subcutaneous infection in a dose of 1×10^5 CFU per mouse. The experiments involving assessment of survival after getting infected with *M. tuberculosis* were terminated on day 111 after infection.

Determination of mycobacterial counts in organs of infected animals

Mycobacterial load in the lung tissue and the spleen was determined on day 50 after infection (5 animals per group). For that the organs were homogenized in 2 mL of saline. The 10-fold dilutions of homogenates were sown on the Middlebrook 7H10 agar (HiMedia Laboratories LLC, USA) (50 µL/Petri dish). After 18–21 days, colonies were enumerated, and CFU/organ were calculated using the following formula: $N = 2N_1 \times D / 0.05$, where N was CFU per organ; N_1 was the number of colonies in the dish; D was dilution.

Statistical analysis

Statistical data processing was performed by two-factor analysis of variance and Tukey's test for multiple comparisons to assess the IgG titer. The Dunn's test as a post-hoc test used after the Kruskal-Wallis test was applied to the ELISpot analysis data and the data on the bacterial load in the lung and spleen of the immunized mice infection with M. tuberculosis. Overall survival was assessed using the Kaplan–Meier estimator. Significance of differences in overall survival was calculated the Mantel–Cox log-rank test. The differences between experimental groups were considered significant at $p < 0.05$. Data analysis and visualization were performed using the GraphPad Prism 10.4.1 software tool (GraphPad Software, USA).

RESULTS

Tuberculosis mRNA vaccine immunogenicity assessment

We assessed the efficacy of two different tuberculosis mRNA vaccines with the antigen sequence MTB-mEp-5-1 based on the linear and circular RNAs. The MTB-mEp-5-1 and circMTB-mEp-5-1 vaccines (Fig. 1A) formulated into lipid nanoparticles (LNPs) were tested for the ability to activate humoral (Fig. 1C) and cell-mediated immune responses (Fig. 1D) compared to the BCG vaccine and control groups (PBS and LNP). When the animals were immunized with the LNPs containing no mRNA, the testing results showed no differences from that of the group of mice receiving PBS.

The highest anti-mycobacterial IgG titers were found in the groups of mice vaccinated with BCG and circMTB-mEp-5-1 ($p < 0.001$ for most dilutions compared to the control; see Table), and IgG levels for BCG and circRNA-MTB-mEp were comparable (Fig. 1C; Table). The increase in IgG titer was less prominent after immunization with MTB-mEp-5-1; the increase in the IgG immunoglobulin titer relative to non-vaccinated animals was reported for the serum dilution 1 : 50 only ($p = 0.001$). The

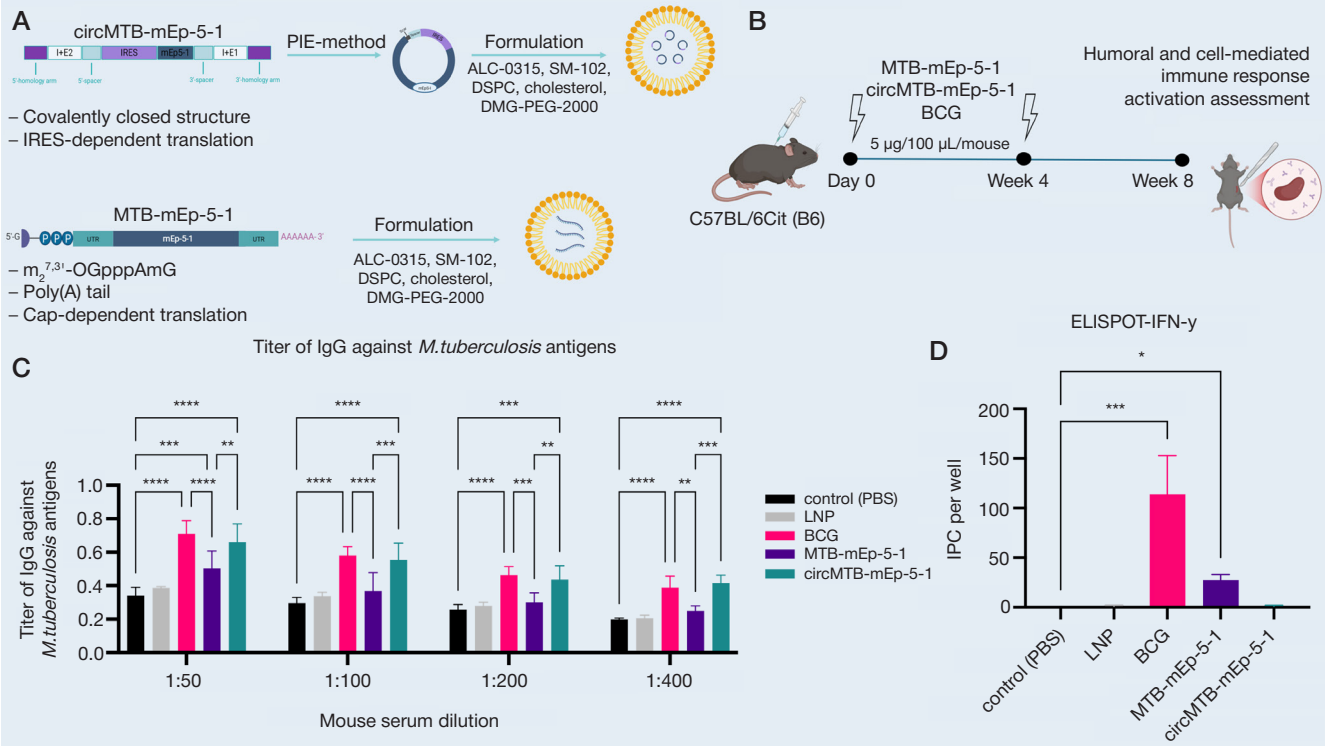

Fig. 1. mRNA vaccine immunogenicity assessment. **A**. Structure of the tuberculosis RNA vaccines with the MTB-mEp-5-1 antigen sequence based on the linear and circular RNAs. **B**. Experimental design scheme. **C**. Titer of IgG immunoglobulins to *M. tuberculosis* antigens in blood serum of mice after immunization with the tuberculosis vaccines. **D**. Differences in IPC counts in the spleen of mice vaccinated with various mRNA vaccine variants. The data are presented as the mean ± standard deviation. Mice per group $n = 5$. * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$; **** — $p < 0.0001$.

anti-mycobacterial IgG levels reported after immunization with MTB-mEp-5-1 were inferior to both circMTB-mEp-5-1 and BCG for almost all serum dilution variants (Fig. 1C; Table). Thus, both mRNA vaccines yielded the humoral immune response to *M. tuberculosis*, but when using the circular RNA-based vaccine, the humoral immune response was stronger and comparable with the immune response observed after BCG vaccination.

To determine the ability of the tuberculosis vaccines based on various RNA platforms to activate specific cell-mediated immune response, we assessed the counts of IFNγ-producing cells (IPC) of the spleen after the mycobacterial sonicate stimulation (Fig. 1D; Table). The highest IPC counts were reported for the group of BCG vaccinated mice (more than 100 spots, $p < 0.001$ compared to the control; Table). Vaccination with MTB-mEp-5-1 also led to the increase in IPC counts relative to the control (more than 20 spots, $p = 0.026$; Table). The mice immunized with circMTB-mEp-5-1 showed no significant differences in IPC counts from the control groups.

Thus, among RNA-based vaccines, only circMTB-mEp-5-1 could yield the humoral immune response comparable to that observed after BCG immunization. However, vaccination with this vaccine did not result in the T-cell immunity development, while immunization with the MTB-mEp-5-1 linear mRNA-based vaccine yielded moderate humoral and cell-mediated immune responses to mycobacterial antigens.

Tuberculosis mRNA vaccine protective effect assessment

For a vaccine, one of the most important criteria is its ability to generate the protective immune response after infection. We conducted comparative assessment of the efficacy of two tuberculosis vaccines based on different RNA platforms by the ability to protect mice after getting infected with *M. tuberculosis* (Fig. 2A). Fig. 2B and the Table show that vaccination with MTB-mEp-5-1 and BCG can ensure the decrease in mycobacterial counts in the lung and spleen on day 50 after infection compared to the control ($p = 0.007$ and $p = 0.006$ for the lung, $p = 0.009$ and $p = 0.022$ for the spleen, respectively). As for mice vaccinated with circMTB-mEp-5-1, no significant decrease in bacterial load in the lung and spleen relative to the control was reported after infection.

When assessing the dynamics of mouse deaths after getting infected, immunization with BCG or MTB-mEp-5-1 ensured survival of 8 and 7 animals out of 10 for 111 days after the *M. tuberculosis* infection ($p < 0.001$; Table). Survival of mice in the control group was 20%. In the group of mice receiving circRNA-MTB-mEp, survival after getting infected showed no differences from that of the control. However, the number of survivors in this group was 50% (Fig. 2C).

Thus, only vaccination with MTB-mEp-5-1 yielded the protective immune response due to the decrease in bacterial load in the lung and spleen and increased survival of the infected mice. The reported protective effect of vaccination with this mRNA vaccine was comparable with that reported after BCG vaccination.

DISCUSSION

In this study, we assessed immunogenic and protective properties of the vaccines against *M. tuberculosis* based on the linear and circular RNAs compared to the BCG vaccine. The findings show that immunization with the MTB-mEp-5-1 linear mRNA-based vaccine yielded the adaptive and protective immune responses, reduced bacterial load in the lung and spleen, and increased survival rate of the infected mice. The MTB-mEp-5-1 immunogenicity was lower than that reported after BCG immunization, but protective efficacy was compared to that observed after BCG.

The circMTB-mEp-5-1 circular RNA-based vaccine turned out to be less effective. Our findings have shown

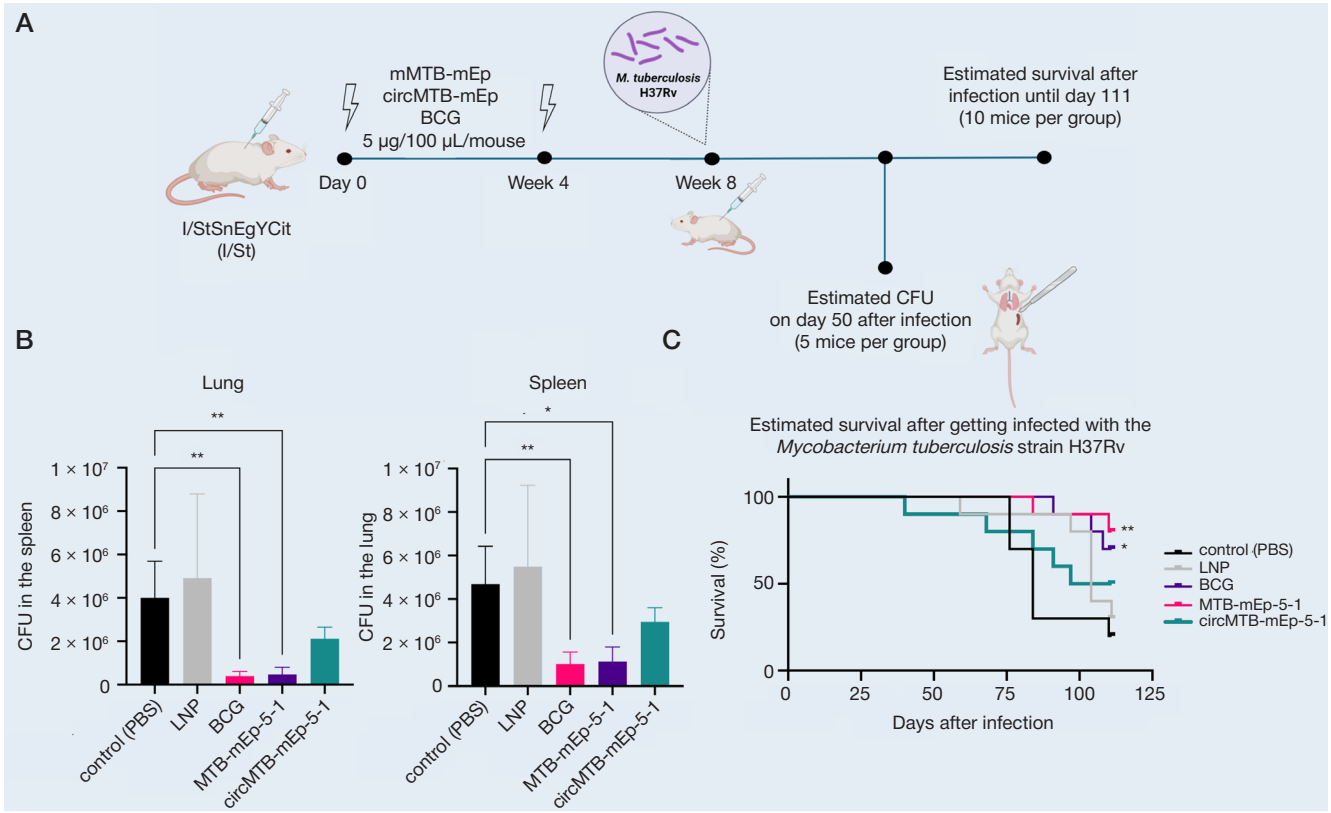

Fig. 2. Protective immune response assessment after immunization with mRNA vaccines **A**. Experimental design scheme. **B**. Bacterial load in the lung tissue and the spleen of immunized mice (n = 5) after the *M. tuberculosis* infection (H37Rv strain). The data are presented as the mean ± standard deviation. **C**. Survival dynamics of mice (n = 10) immunized with tuberculosis vaccines after the *M. tuberculosis* infection (H37Rv strain). * — $p < 0.05$; ** — $p < 0.01$.

that circMTB-mEp-5-1 can induce the pronounced humoral immune response comparable to that observed after BCG vaccination. However, immunization with circMTB-mEp-5-1 yielded no IFNγ-mediated T cell-mediated response and did not ensure protection of animals against the *M. tuberculosis* infection. These results show that yielding humoral immunity only is insufficient to ensure protection against pathogen, it is also necessary to yield T cell-mediated immunity, which is consistent with the results of the earlier studies of the nucleic acid-based tuberculosis vaccines [2].

Apparently, the differences in mRNA vaccine efficacy are associated with the mechanisms underlying the dynamics of antigen translation. Circular RNAs differ from linear RNAs by increased stability [7]. High circRNA stability contributes to prolonged antigen production, which can result in the pronounced humoral immune response, but due to IRES the antigen translation is considerably lower, than the speed of translation through the cap-dependent pathway with the linear RNA [18], which results in low antigen expression that can be not enough to develop the cell-mediated and protective immunity. The role of the T cell-mediated immunity is important to form granulomas. That is why in the earlier studies the main efforts were focused on the development of vaccines yielding the strong cell-mediated immune response [19]. However, a number of recent rodent, non-human primate, and human studies have shown that humoral immunity induction is important for vaccine efficacy [20]. This is in line with our data obtained for the linear RNA than induced moderate humoral

Table. Summary of study results

IgG titer (mean ± standard deviation, OD = A450)					
Dilution	BCG	MTB-mEp-5-1	circRNA-MTB-mEp	LNP	PBS
1 : 50	0.71 ± 0.08	0.50 ± 0.10	0.66 ± 0.11	0.39 ± 0.01	0.34 ± 0.05
1 : 100	0.58 ± 0.05	0.37 ± 0.11	0.56 ± 0.10	0.34 ± 0.02	0.30 ± 0.03
1 : 200	0.46 ± 0.05	0.30 ± 0.06	0.44 ± 0.08	0.28 ± 0.02	0.26 ± 0.03
1 : 400	0.39 ± 0.07	0.25 ± 0.03	0.42 ± 0.05	0.21 ± 0.02	0.20 ± 0.01
IPCs per well (mean ± standard deviation)					
	114.00 ± 38.99	27.60 ± 5.41	1.00 ± 1.00	1.00 ± 1.00	0.20 ± 0.45
CFU in the lung (mean ± standard deviation)					
	$4.00 \times 10^5 \pm 2.13 \times 10^5$	$4.72 \times 10^5 \pm 3.44 \times 10^5$	$2.12 \times 10^6 \pm 5.30 \times 10^5$	$4.91 \times 10^6 \pm 3.89 \times 10^6$	$4.01 \times 10^6 \pm 1.69 \times 10^6$
CFU in the spleen (mean ± standard deviation)					
	$1.01 \times 10^6 \pm 5.52 \times 10^5$	$1.13 \times 10^6 \pm 6.78 \times 10^5$	$2.95 \times 10^6 \pm 6.47 \times 10^5$	$5.49 \times 10^6 \pm 3.75 \times 10^6$	$4.69 \times 10^6 \pm 1.74 \times 10^6$
Median survival					
	–	–	94	104	84

and cell-mediated immune responses that ensured the development of protective response.

One more possible explanation of developing stronger humoral immunity after immunization with circMTB-mEp-5-1 is possible excess innate immunity activation after vaccination. Along with the viral IRES sequence, circular RNAs also comprise bacterial sequences: the intron-exon regions being the essential elements for RNA circularization, when the self-splicing group 1 introns are used [21]. The study conducted by Chen et al. has shown that the exogenous circular RNA obtained using the self-splicing group 1 introns can activate the innate immune response receptors (RIG-1, MDA5, OAS1, OASL), in contrast to the endogenous circular RNA obtained using human introns deprived of the capability of autocatalytic splicing, but capable of forming circular RNAs in the cell using a spliceosome. The endogenous circular RNA is not identified as a foreign one, since it is associated with many RNA-binding proteins [22]. Due to its structure, the exogenous circular RNA can activate the innate immunity receptors, thereby contributing to the higher reactogenicity of the circular RNA-based vaccines compared to the linear RNA-based one. Activation of the innate immunity receptors, such as RIG-1 and MDA5, results in IFN-I production [23], which can cause activation of humoral immunity, intense B cell-mediated response [24], as well as inhibition of the T cell-mediated response [25].

However, the circular RNA-based vaccine is likely to be effective against viral infections, in which it is more important to generate a strong humoral immune response. Perhaps in the future it will be a promising avenue to combine the circular RNA- and linear RNA-based vaccines in the same formulation or different prime-boost strategies in order to enhance both humoral and T cell-mediated immune responses and ensure more effective protective immunity.

When constructing vaccines against *M. tuberculosis*, it is necessary to consider one more problem: the microorganism complexity. Given the fact that *M. tuberculosis* comprises about 4000 genes [26], the use of strategies that are used to develop the viral mRNA-based vaccines can hardly be applicable to the mRNA-based tuberculosis vaccines. The M. tuberculosis genetic complexity is in contrast to the relatively simple structure of viral genomes and is further enhanced due to unusual genetic diversity reported for various mycobacterial strains [27]. Today, there is no evidence that one particular antigen or a limited number of antigens can play a crucial role in ensuring the protection.

It is likely that in the future the use of the heterologous boost immunization to enhance the BCG vaccine effect will become an important component of complex TB vaccination strategies. Heterologous revaccination can be performed in infancy or adolescence, when the BCG effect starts to wane. The mRNA-based vaccines are at the forefront of the research focused on assessing the possibility of enhancing cell-mediated immunity against TB through heterologous boost immunization. Despite the fact that the infection limitation for TB prevention can be a realistic first step, the ultimate goal should be to use the heterologous boost regimen to create either pre-exposure vaccines preventing the development of infection and disease, or post-exposure vaccine capable of stopping disease reactivation in individuals with latent infection.

CONCLUSIONS

The data obtained on immunogenicity and protective efficacy of the linear RNA-based vaccines confirm that these are promising in terms of using as vaccines against M. tuberculosis. The circMTB-mEp-5-1 circular RNA-based vaccine is still inferior in efficacy to that based on the linear RNA, despite the fact that it ensures a rather strong humoral immune response. Further optimization is required to enhance cell-mediated and protective immune responses.

References

1. Global Tuberculosis Report 2024. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024 (access date: 19.05.2025).
2. Kazakova A, Zhelnov P, Sidorov R, Rogova A, Vasileva O, Ivanov R, et al. DNA and RNA vaccines against tuberculosis: a scoping review of human and animal studies. Frontiers in Immunology. 2024; 15: 1457327.
3. Working Group for New TB Vaccines. Available from: https://newtbvaccines.org/ (access date: 12.07.2025).
4. Baden LR, El Sahly H.M, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. New England Journal of Medicine. 2021; 384 (5): 403–16.
5. Khlebnikova A, Kirshina A, Zakharova N, Ivanov R, Reshetnikov V. Current Progress in the Development of mRNA Vaccines Against Bacterial Infections. International Journal of Molecular Sciences. 2024; 25 (23): 13139.
6. Weng Y, Li C, Yang T, Hu B, Zhang M, Guo S, et al. The challenge and prospect of mRNA therapeutics landscape. Biotechnology Advances. 2020; 40: 107534.
7. Liu X, Zhang Y, Zhou S, Dain L, Mei L, Zhu G. Circular RNA: An emerging frontier in RNA therapeutic targets, RNA therapeutics, and mRNA vaccines. Journal of Controlled Release. 2022; 348: 84–94.
8. Hwang HJ, Kim YK. Molecular mechanisms of circular RNA translation. Experimental & molecular medicine. 2024; 56 (6): 1272–80.
9. Margvelani G, Maquera KAA, Welden JR, Rodgers DW, Stamm S. Translation of circular RNAs. Nucleic acids research. 2025; 53 (1): gkae1167.
10. Zhou J, Ye T, Yang Y, Li E, Zhang K, Wang Y, et al. Circular RNA vaccines against monkeypox virus provide potent protection against vaccinia virus infection in mice. Molecular Therapy. 2024; 32 (6): 1779–89.
11. Qu L, Yi Z, Shen Y, Lin L, Chen F, Xu Y, et al. Circular RNA vaccines against SARS-CoV-2 and emerging variants. Cell. 2022; 185 (10): 1728–44.
12. Radaeva TV, Nikonenko BV, Mischenko VV, Averbakh MM, Apt AS. Direct comparison of low-dose and Cornell-like models of chronic and reactivation tuberculosis in genetically susceptible I/St and resistant B6 mice. Tuberculosis. 2005; 85 (1–2): 65–72.
13. Reshetnikov V, et al. The Candidate Anti-Tuberculosis MRNA Vaccine Immunogenicity and Reactogenicity Dependency on the Animal's Sex and the Vaccine Dose. Bull Russ State Med Univ. 2024; 5: 25–31.
14. Kozlova A, Pateev I, Shepelkova G, Vasileva O, Zakharova N, Yeremeev V, et al. A Cap-Optimized MRNA Encoding Multiepitope Antigen ESAT6 Induces Robust Cellular and Humoral Immune Responses Against Mycobacterium tuberculosis. Vaccines. 2024; 12 (11): 1267.
15. Reshetnikov V, Terenin I, Shepelkova G, Yeremeev V, Kolmykov S, Nagornykh M, et al. Untranslated region sequences and the efficacy of mRNA vaccines against tuberculosis. International Journal of Molecular Sciences. 2024; 25 (2): 888.
16. Avdienko VG, Babayan SS, Bocharova IV. Characteristics and application of monoclonal antibodies to Mycobacterium kansasii.

CTRI Bulletin. 2024; 8 (4): 51–60.
17. Nikonenko BV, Apt AS, Mezhlumova MB, Avdienko VG, Yeremeev VV, Moroz AM. Influence of the mouse Bcg Tbc-1 and xid genes on resistance and immune responses to tuberculosis infection and efficacy of bacille Calmette–Guérin (BCG) vaccination. Clinical and Experimental Immunology. 2003; 104 (1) 37–43.
18. Yang Y, Wang Z. IRES-mediated cap-independent translation, a path leading to hidden proteome. Journal of molecular cell biology. 2019; 11 (10): 911–9.
19. Lai R, Ogunsola AF, Rakib T, Behar SM. Key advances in vaccine development for tuberculosis—success and challenges. NPJ vaccines. 2023; 8 (1): 158.
20. Rijnink WF, Ottenhoff TH, Joosten SA. B-cells and antibodies as contributors to effector immune responses in tuberculosis. Frontiers in immunology. 2021; 12: 640168.
21. Obi P, Chen YG. The design and synthesis of circular RNAs. Methods. 2021; 196: 85–103.
22. Chen YG, Kim MV, Chen X, Batista PJ, Aoyama S, Wilusz JE. Sensing self and foreign circular RNAs by intron identity. Molecular cell. 2017; 67 (2): 228–38.
23. Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA sensing. Nature Reviews Immunology. 2020; 20 (9): 537–51.
24. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity. 2001; 14 (4): 461–70.
25. Crouse J, Kalinke U, Oxenius A. Regulation of antiviral T cell responses by type I interferons. Nature Reviews Immunology. 2015; 15 (4): 231–42.
26. Cole St, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature. 1998; 396 (6707): 190.
27. Gagneux S, Small PM. Global phylogeography of Mycobacterium tuberculosis and implications for tuberculosis product development. The Lancet infectious diseases. 2007; 7 (5): 328–37.

Литература

1. Global Tuberculosis Report 2024. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024 (дата обращения: 19.05.2025).
2. Kazakova A, Zhelnov P, Sidorov R, Rogova A, Vasileva O, Ivanov R, et al. DNA and RNA vaccines against tuberculosis: a scoping review of human and animal studies. Frontiers in Immunology. 2024; 15: 1457327.
3. Working Group for New TB Vaccines. Available from: https://newtbvaccines.org/ (дата обращения: 12.07.2025).
4. Baden LR, El Sahly H.M, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. New England Journal of Medicine. 2021; 384 (5): 403–16.
5. Khlebnikova A, Kirshina A, Zakharova N, Ivanov R, Reshetnikov V. Current Progress in the Development of mRNA Vaccines Against Bacterial Infections. International Journal of Molecular Sciences. 2024; 25 (23): 13139.
6. Weng Y, Li C, Yang T, Hu B, Zhang M, Guo S, et al. The challenge and prospect of mRNA therapeutics landscape. Biotechnology Advances. 2020; 40: 107534.
7. Liu X, Zhang Y, Zhou S, Dain L, Mei L, Zhu G. Circular RNA: An emerging frontier in RNA therapeutic targets, RNA therapeutics, and mRNA vaccines. Journal of Controlled Release. 2022; 348: 84–94.
8. Hwang HJ, Kim YK. Molecular mechanisms of circular RNA translation. Experimental & molecular medicine. 2024; 56 (6): 1272–80.
9. Margvelani G, Maquera KAA, Welden JR, Rodgers DW, Stamm S. Translation of circular RNAs. Nucleic acids research. 2025; 53 (1): gkae1167.
10. Zhou J, Ye T, Yang Y, Li E, Zhang K, Wang Y, et al. Circular RNA vaccines against monkeypox virus provide potent protection against vaccinia virus infection in mice. Molecular Therapy. 2024; 32 (6): 1779–89.
11. Qu L, Yi Z, Shen Y, Lin L, Chen F, Xu Y, et al. Circular RNA vaccines against SARS-CoV-2 and emerging variants. Cell. 2022; 185 (10): 1728–44.
12. Radaeva TV, Nikonenko BV, Mischenko VV, Averbakh MM, Apt AS. Direct comparison of low-dose and Cornell-like models of chronic and reactivation tuberculosis in genetically susceptible I/St and resistant B6 mice. Tuberculosis. 2005; 85 (1–2): 65–72.
13. Reshetnikov V, et al. The Candidate Anti-Tuberculosis MRNA Vaccine Immunogenicity and Reactogenicity Dependency on the Animal's Sex and the Vaccine Dose. Bull Russ State Med Univ. 2024; 5: 25–31.
14. Kozlova A, Pateev I, Shepelkova G, Vasileva O, Zakharova N, Yeremeev V, et al. A Cap-Optimized MRNA Encoding Multiepitope Antigen ESAT6 Induces Robust Cellular and Humoral Immune Responses Against Mycobacterium tuberculosis. Vaccines. 2024; 12 (11): 1267.
15. Reshetnikov V, Terenin I, Shepelkova G, Yeremeev V, Kolmykov S, Nagornykh M, et al. Untranslated region sequences and the efficacy of mRNA vaccines against tuberculosis. International Journal of Molecular Sciences. 2024; 25 (2): 888.
16. Avdienko VG, Babayan SS, Bocharova IV. Characteristics and application of monoclonal antibodies to Mycobacterium kansasii. CTRI Bulletin. 2024; 8 (4): 51–60.
17. Nikonenko BV, Apt AS, Mezhlumova MB, Avdienko VG, Yeremeev VV, Moroz AM. Influence of the mouse Bcg Tbc-1 and xid genes on resistance and immune responses to tuberculosis infection and efficacy of bacille Calmette–Guérin (BCG) vaccination. Clinical and Experimental Immunology. 2003; 104 (1) 37–43.
18. Yang Y, Wang Z. IRES-mediated cap-independent translation, a path leading to hidden proteome. Journal of molecular cell biology. 2019; 11 (10): 911–9.
19. Lai R, Ogunsola AF, Rakib T, Behar SM. Key advances in vaccine development for tuberculosis—success and challenges. NPJ vaccines. 2023; 8 (1): 158.
20. Rijnink WF, Ottenhoff TH, Joosten SA. B-cells and antibodies as contributors to effector immune responses in tuberculosis. Frontiers in immunology. 2021; 12: 640168.
21. Obi P, Chen YG. The design and synthesis of circular RNAs. Methods. 2021; 196: 85–103.
22. Chen YG, Kim MV, Chen X, Batista PJ, Aoyama S, Wilusz JE. Sensing self and foreign circular RNAs by intron identity. Molecular cell. 2017; 67 (2): 228–38.
23. Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA sensing. Nature Reviews Immunology. 2020; 20 (9): 537–51.
24. Le Bon A, Schiavoni G, D'Agostino G, Gresser I, Belardelli F, Tough DF. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. Immunity. 2001; 14 (4): 461–70.
25. Crouse J, Kalinke U, Oxenius A. Regulation of antiviral T cell responses by type I interferons. Nature Reviews Immunology. 2015; 15 (4): 231–42.
26. Cole St, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature. 1998; 396 (6707): 190.
27. Gagneux S, Small PM. Global phylogeography of Mycobacterium tuberculosis and implications for tuberculosis product development. The Lancet infectious diseases. 2007; 7 (5): 328–37.

MICROBIOLOGICAL ANALYSIS AND IDENTIFICATION OF PATHOGENS IN ORTHOPEDIC THEATRES: AL-NASSIRIYAH CITY'S STUDY

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Bacteria found in the operating room can lead to surgical site and hospital-acquired infections. This study was conducted in Imam Hussein Hospital in Nasiriyah to investigate the contamination levels in the operating rooms. The main goal of the research was to identify the bacteria responsible for contamination and the factors contributing to it. The study also aimed to map these microorganisms' distribution across different operating room areas and their antibiotic resistance pattern using microbiological standards. We gathered 1358 samples for analysis from surfaces and objects in the operating room. The results showed that 3.1% tested positive for bacteria, and 96.9% were negative cultures. Six types of pathogenic bacteria have been identified; Coagulase-negative staphylococci 14.3%, *Staphylococcus aureus* 11.9%, *Pseudomonas aeruginosa* 19.1%, *E. coli* 21.4%, *Bacillus spp.* 11.9%, and Enterobacter spp. 21.4%. We observed moderate to high resistance pattern to amoxicillin and ampicillin, Cefaclor, Cefuroxime, Cefadroxil, Erythromycin. The highest resistance pattern was detected in *P. aeruginosa* isolates followed by *E. coli*, it showed different resistance patterns to 14 antibiotics showing susceptibility to Amikacin only. Conclusion: the study at Imam Hussein Hospital found a generally low but notable level of bacterial contamination in orthopedic operating theaters, with specific pathogens posing risks to patients. These findings align with global data, underscoring common challenges in maintaining sterile surgical environments. Identifying contamination hotspots and patterns over time highlights the need for targeted interventions and continuous monitoring.

Keywords: *Pseudomonas aeruginosa*, operating room areas, antibiotic resistance

Compliance with ethical standards: the study was conducted in accordance with the standards of good clinical practice and evidence-based medicine.

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МИКРОБИОЛОГИЧЕСКИЙ АНАЛИЗ И ИДЕНТИФИКАЦИЯ ПАТОГЕНОВ ВО ВРЕМЯ ОРТОПЕДИЧЕСКИХ ОПЕРАЦИЙ: ИССЛЕДОВАНИЕ В Г. ЭН-НАСИРИЯ

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Бактерии, встречающиеся в операционных, могут приводить к хирургическим и внутрибольничным инфекциям. Целью работы было идентифицировать бактерии, ответственные за обсеменение, определить факторы, способствующие обсеменению, а также картировать распределение этих микроорганизмов по различным зонам операционного блока и определить характер их устойчивости к противомикробным средствам с использованием микробиологических стандартов. Было отобрано 1358 образцов с поверхностей и предметов в операционной. В 3,1% случаев результаты анализа на наличие бактерий были положительными, а в 96,9% случаев имело место отсутствие роста бактерий. Идентифицированы патогенные бактерии шести типов: коагулазонегативные стафилококки (14,3%), *Staphylococcus aureus* (11,9%), *Pseudomonas aeruginosa* (19,1%), *E. coli* (21,4%), *Bacillus spp*. (11,9%) и *Enterobacter spp*. (21,4%). Выявлена умеренная и сильная устойчивость к амоксициллину и ампициллину, цефаклору, цефуроксиму, цефадроксилу, эритромицину. Самая высокая устойчивость выявлена у изолятов *P. aeruginosa*. На втором месте — *E. coli*, продемонстрировавшая разную устойчивость к 14 противомикробным средствам и чувствительность только к амикацину. Таким образом, исследование, проведенное в Больнице Имама Хусейна, выявило в целом низкий, но заметный уровень обсеменения ортопедических операционных опасными для пациентов специфическими патогенами. Полученные результаты согласуются с глобальными данными, что говорит об универсальности проблемы обеспечения стерильности операционных. Постепенное выявление очагов и закономерностей обсеменения подчеркивает необходимость применения целенаправленных мер и постоянного наблюдения.

Ключевые слова: *Pseudomonas aeruginosa*, зоны операционного блока, устойчивость к противомикробным средствам

Соблюдение этических стандартов: исследование проведено в соответствии со стандартами добросовестной клинической практики и доказательной медицины.

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A critical factor in the occurrence of hospital-acquired infections (HAI) is the existence of germs in the room. The clinical implications of this finding are significant, not only for the patient but also for the surgical team that is caring for them [1, 2]. According to statistics from the Centers for Disease Control and Prevention (CDC), nosocomial infections impact around 1.7 million people annually. They are responsible for 99.000 fatalities [3]. Orthopedic surgery, especially elective treatments such as joint arthroplasty and hand surgery, reduces the risk of postoperative infection [4]. Orthopaedic surgery implements more stringent sterile protocols compared to other surgical procedures. Although the measurement technique used to assess the NI risk index may vary, the incidence might fluctuate greatly [5].

On the other hand, NI continues to be a source of worry because of the significant morbidity that results from it and the

extended hospital stays, readmissions, and revision operations that lead to a rise in medical treatment expenses [6, 7]. According to the data, due to bacterial NI, approximately 1718 total operations were performed in Nasiriyah during the past two years (2021–2023) for hip, knee, internal fixation, surgery, hip, and hand surgery. It should come as no surprise that these concerning figures have prompted several attempts by medical experts to identify and minimize NI origins and associated risks. Research has revealed many sources linked to the spread of pathogenic microorganisms, such as air, hospital areas, nitrogen gas freezers, staff clothing, and even keeping sterile plates open for an extended period [8, 9].

It has been determined that the operating room pollution results from several distinct reservoirs. These storage containers include unprocessed air, ventilated spaces, and antiseptic fluids; drainage of wounds; patient and gathering bag travel; surgical group; degree of inside traffic; theatre dress; foot wares; gloves and hands; use of inadequately sterilized supplies; polluted surroundings; and grossly polluted surfaces [10, 11]. By appropriately implementing infection control procedures, it is possible to avoid microbial contamination in the operating room. Reducing airborne bacteria in the operating room by about thirteen times, for instance, would result in a 50% reduction in wound contamination this mostly depends on the frequent fumigation of operation theater (OT) and improving cleaning and disinfection procedures.[12, 13].

In addition, research has shown that mobile devices, which medical professionals use throughout the hospital, are a prominent origin of pollution inside the operating room. Several studies have shown that disease-causing microbes, such as coagulase-negative staphylococci (CoNS), *S. aureus*, and *Acetinobacter*, utilize mobile phones [14, 15].

Several preventable causes of NI have been identified. If the proper treatments are implemented, the incidence of NI may be decreased. There are many primary focus areas of concern, including patients, surgeons, nurses, the atmosphere of the operating room, and the equipment. Although several methods have been established to reduce the risk of infection after implant surgery, infection is still a potential. To reduce the number of infections that arise in the operating room, engaging in practices such as practicing hand hygiene, providing prophylactic antibiotics in an acceptable manner and at the proper dose, donning surgical clothing, and reducing staff mobility is important. Surgical site infections are the third most frequently reported nosocomial infection (about 14–16%) . Given that antibiotic resistance has become a worldwide issue with corresponding consequences, the operating rooms serve as both a breeding ground and a hub for the spread of multidrug-resistant (MDR) microorganisms across the hospital setting. In developed and developing nations, the prevalence of nosocomial infections was linked directly or indirectly to bacterial contamination has been rising despite the high degree of relative hygiene and cleanliness as well as basic understanding of infection control with preventive techniques [16, 17].

It becomes essential to conduct surveillance within the unit to produce the vastly needed epidemiological data, which serves as a model for the creation of infection control and prevention policies. Bone and joint infection are considered a major cause of morbidity ' emotional stress, and enhanced mortality, in addition to significant economic loss ,and so one can imagine the magnitude of the problem. The purpose of this study was to examine the degree of contamination in the operating rooms at Imam Hussein Hospital in Nasiriyah. Finding the bacteria causing contamination and the related factors was the primary objective of the study. Using microbiological criteria, the study also sought to map the spread of these microbes throughout several operating room locations and their pattern of antibiotic resistance. We made the decision to evaluate the degree of bacterial contamination in our hospital's Orthopedic Theatres rooms in light of this information.

METHODS

Imam Hussein Hospital, located in the city of Nasiriyah under study, contained three distinct operating rooms, distributed as three rooms designated for general surgery, two for urology, and three rooms for orthopedic operation theatres between February 2022 and May 2024. We assessed the microbial contamination at orthopedic theatres, within the hospital approximately four to five times per month. To perform the swab, sterile swabs were soaked in sterile normal saline, then instantly & severally rolled onto floor surfaces, doors, telephone hands, walls, washbasins, and medical tools in the operating room and the intensive care unit (ICU) before and after surgery, then the samples were properly labeled before transportation to the to be processed in Laboratory. After sample collection, all swabs were immediately cultured using the sectioning technique on enriched medium blood agar and CLED agar [18] (Fig. 1).

In accordance with CSLI guidelines on Mueller Hinton agar, antibiotic susceptibility testing was conducted using the disc diffusion technique (Fig. 2). The 15 tested antibiotic discs were Ampicillin (AM), Amoxicillin (AMX), Amoxicillin/Clavulate (AMC), Cefaclor (CEC), Cefadroxil (CFR), Cefuroxime (CXM), Ceftriaxon (CRO), Ceftazidime (CAZ), Cefotaxim (CTX), Cefixim (CFM), Erythromycin (ERY), Ciprofloxacin (CIP), Levofuxacin (LE), Gentamycin (GM) Amikacin (AK) (Fig. 3). Following the guidelines, the zone of growth inhibition diameter of the bacterial isolates was measured and interpreted [19].

Statistical analysis

Values expressed as frequencies and percentages were analyzed using the statistical software SPSS version 17.0. Statistical analysis of the data was done considering an alpha error of 0.05 with a 95% confidence interval. Data are presented by mean ± SD. Frequency and percentage are used to describe demographic and baseline factors. Chi-square test was used for the above comparisons when the test assumptions were met, otherwise T- test method test was used.

RESULTS

For microbiological analysis, one thousand three hundred fifty-eight swabs were taken from the orthopedic surgery rooms between February 2022 and May 2024. Following the inoculation and cultivation of the swabs under aerobic and anaerobic conditions, it was discovered that 42 (3.09%) of the swabs had positive growth, while the negative culture was 1316 (96.90%).

The temporal distribution of positive culture shows a high pattern in the months (February, March, April), while there was generally a decrease in May.

A high incidence of positive cultures was detected in those swabs obtained from the couch of operating room 12 (28.57%), floor 11 (26.19%), and wall 9 (21.43%). A low incidence of positive cultures was recorded in those swabs taken from section parts 5 (11.91%), antiseptic solutions 3 (7.14%), and anesthetic trollies 2(4.76%), swabs from gauze

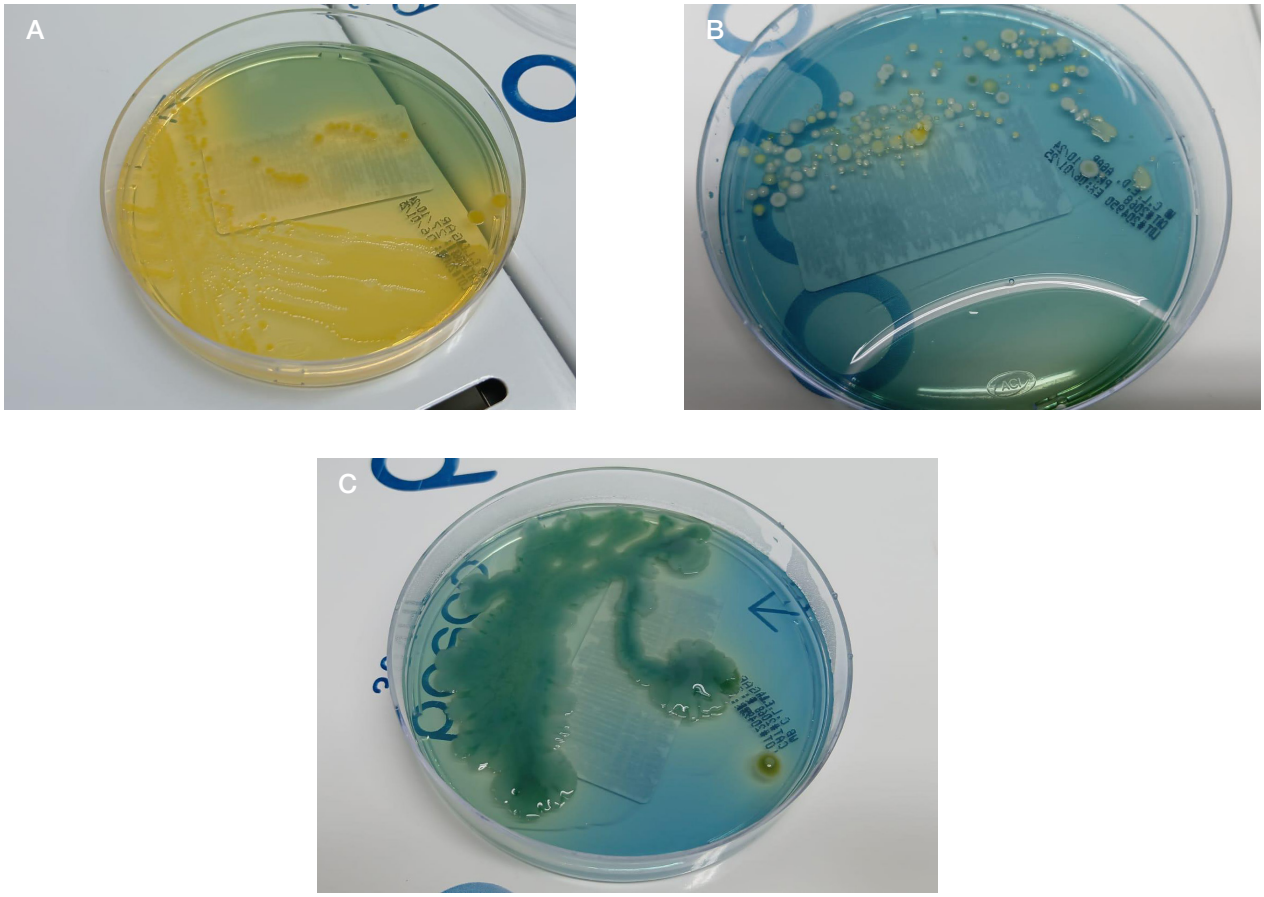

Fig. 1. A. Gram –ve bacteria appearance on CLED media. **B**. Gram +ve bacteria appetence on CLED media. **C**. Characteristic appetence of *P. aeruginosa* isolates

(0%) and surgical instruments (0%) show no growth of bacteria %) (Fig. 3). A highly significant difference (*P*-value < 0.001) was detected in bacterial contamination rates across different swab sources, where the contamination in couch, floor, and wall was significantly more contaminated than other areas.

Six types of pathogenic bacteria have been identified in orthopedic operating rooms as follows: CoNS 6 (14.29%), *S. aureus* 5 (11.90%), *P. aeruginosa* 8 (19.05%), *E. coli* 9 (21.43%), *Bacillus spp*. 5 (11.90%), and *Enterobacter spp*. 9 (21.43%). There is no statistically significant difference in the frequency distribution of the bacterial species (Table 1).

We observed moderate to high resistance pattern (Table 2) to amoxicillin and ampicillin, Cefaclor, Cefuroxime, Cefadroxil, Erythromycin. All the detected organisms showed highest resistances to both amoxicillin and ampicillin, this resistance pattern were decreased upon adding clavulate to amoxicillin.

S. aureus demonstrated the highest resistance to Ampicillin and amoxicillin (*n* = 5), followed by Amoxicillin/ Clavulate and Erythromycin (*n* = 3), Cefaclor (*n* = 2), only one isolate was resistant to Cefadroxil, Ceftazidime, Cefotaxim, Cefixim, and Gentamycin, While all the isolates were susceptible to the rest of the tested antibiotics. The highest resistance pattern was detected in *P. aeruginosa* isolates followed by *E. coli*, it shoed different resistance patterns to 14 antibiotics showing susceptibility to Amikacin only. The lowest resistant patterns were detected among CoNS, *Enterobacter spp.* & *Bacillus spp*. Which showed resistance to (4, 5, 4) antibiotics, respectively. Resistance rates differ significantly (*P*-value < 0.05) across antibiotics with the highest resistance battern to AM, AMX, ERY, while the least resistance to LE, AK, CFM. Also, the resuts shoed that, *E. coli* and *P. aeruginosa* were significantly resistance to antibiotics than other bacteria (*P*-value < 0.05).

DISCUSSION

It is possible for patients and their families to face severe consequences as a result of postoperative infections that are caused by microbial contamination in operating rooms. Investigating any suspected case of hospital-acquired infection (HAI) involves the collection of cultures from several regions of the patient's body, as well as from other patients, staff members, and the surrounding environment [20]. When trying to gather useful data, it is vital to pick the specimens that will be cultivated with great care. Infections are responsible for extending hospital admissions, causing long-term impairment, increasing resistance to antimicrobials, causing avoidable deaths, and representing a large additional cost burden for

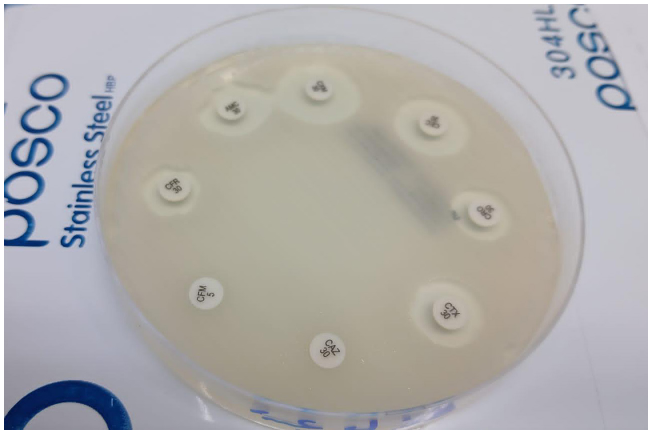

Fig. 2. Bacterial culture showing resistance to both Ceftazidime (CAZ), Cefixim(CFM)

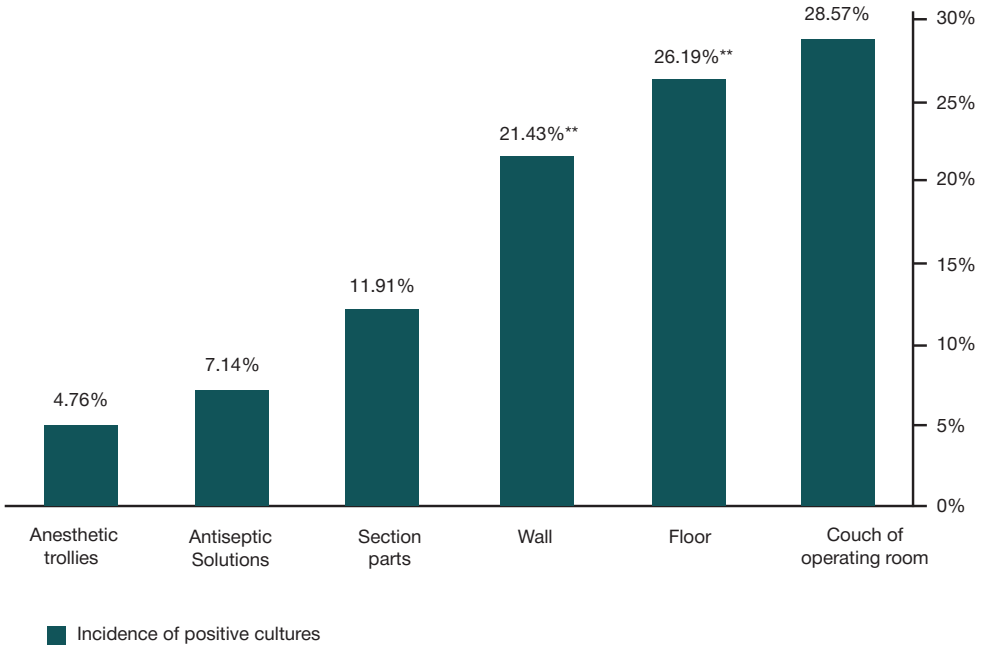

Fig. 3. Visualization of the Frequency of Positive Microbial Cultures in Operating Rooms Broken Down by Sample Source. ** — indicates statically significant difference, *P*-value < 0.001

health systems. Therefore, the solution is a well-implemented infection control program that may increase staff education and accountability.

Additionally, research should be conducted to adapt and validate surveillance procedures based on developing nations' realities to attain acceptable performance. This can potentially minimize the number of HAIs by approximately one-third [21]. The environmental disinfection and instrument sterilization procedures undoubtedly demand the most stringent monitoring out of all the procedures and regulations.

The study identified six types of pathogenic bacteria in the positive swabs: CoNS (14.29%), *S. aureus* (11.90%), *P. aeruginosa* (19.05%), *E. coli* (21.43%), *Bacillus spp*. (11.90%), and *Enterobacter spp*. (21.43%). These pathogens are well-known culprits in hospital-acquired infections, and their presence in operating theaters is a matter of concern. *S. aureus* is notorious for its role in postoperative wound infections. It can be challenging to treat due to its resistance to multiple antibiotics. The relatively high incidence of *E. coli* and *Enterobacter spp.*, which accounted for 21.43% of the identified pathogens, highlights the potential for contamination from sources like surgical instruments or healthcare workers' hands, emphasizing the need for strict sterilization protocols.

The distribution of positive cultures within the orthopedic operating rooms shows a concerning pattern, with the highest contamination rates observed on the couch (28.57%), floor (26.19%), and walls (21.43%). This suggests that these surfaces, which are frequently in contact with patients, staff, and equipment, are critical contamination points. The presence of pathogens on the couch, where patients lie during surgery, directly threatens patient safety, potentially leading to surgical site infections. The significant contamination of the floor and walls further emphasizes the need for rigorous cleaning protocols and the regular disinfection of all surfaces within the operating room. In contrast, a lower incidence of positive cultures were found on section parts (11.91%), antiseptic solutions (7.14%), and anesthetic trollies (4.76%). At the same time, no bacterial growth was detected on gauze or surgical instruments. The absence of contamination on surgical instruments and gauze is a positive finding, indicating that sterilization procedures for these critical items effectively prevent the transmission of pathogens. This aligns with best infection control practices, prioritizing the sterility of instruments used in invasive procedures. A study cited by [22] showed that operating rooms harbor bacteria, S.aureus, also found in this study with a prevalence rate of around 15% in surgical settings — slightly higher than the 10.34% detected in AL Nassiriyah.

Variations in how hospitals handle sanitation and infection control measures in different regions could explain the differences observed.

A relevant study [23] focused on contamination in clean orthopedic procedures, highlighting Coagulase *Staphylococci* and *Enterobacter spp,* as the types of bacteria. These findings align with the study emphasizing the prevalence of

Table 1. Distribution of bacterial isolates in operation surgical rooms

Species isolated	No. of isolated	*n* (%)
CoNS	6	–14.29%
S. aureus	5	–11.90%
P. aeruginosa	8	–19.05%
E. coli	9	–21.43%
Bacillus spp.	5	–11.90%
Enterobacter spp.	9	–21.43%
Total	42	100%
P-value		0.766

Table 2. Antibiotic resistance patterns of the detected bacterial isolates

Enterobacter spp.	*Bacillus spp.*	*E. coli*	*P. aeruginosa*	*S. aureus*	CoNS	Antibiotic
4	3	5	8	5	4	AM
2	2	6	8	5	3	AMX
1	0	3	6	3	0	AMC
0	1	5	6	2	0	CEC
0	0	6	7	1	0	CFR
0	1	4	7	0	1	CXM
0	0	1	3	0	0	CRO
0	0	1	2	1	0	CAZ
0	0	1	3	1	0	CTX
0	0	0	1	1	0	CFM
1	1	3	6	3	2	ERY
0	0	1	2	0	0	CIP
0	0	0	1	0	0	LE
0	0	2	2	1	0	GM
0	0	1	0	0	0	AK

Note: mpicillin (AM), Amoxicillin (AMX), Amoxicillin/Clavulate (AMC), Cefaclor (CEC), Cefadroxil (CFR), Cefuroxime (CXM), Ceftriaxon (CRO), Ceftazidime (CAZ), Cefotaxim (CTX), Cefixim (CFM), Erythromycin (ERY), Ciprofloxacin (CIP), Levofuxacin (LE), Gentamycin (GM), Amikacin (AK).

Coagulase *Staphylococci* and *Enterobacter* species. The study emphasized the role of laminar ventilation systems in preventing contamination and the impact of bacteria, indicating improvements in operating rooms in AL Nassiriyah.

Escherichia coli and *P. aeruginosa* raise concerns about procedure environment sterility. *P. aeruginosa,* known for its resistance and link to hospital-acquired infections, was found in 17.24% of samples. This contrasts with a study [24] reporting *Pseudomonas* prevalence in surgical settings, highlighting a notable increase.

This suggests that the methods used to control infections in AL Nassiriyah might not have been effective. Likewise, *Escherichia coli* was found in 24.14% of the samples, which is much higher than in studies like the one referenced by [25], where *E. coli* was found in more than 10% of samples. This difference highlights the need for hygiene and sterilization practices to prevent contaminations. The impact of these contaminants on patients cannot be overstated. Infections after surgery can lead to procedure complications, resulting in longer hospital stays, more surgeries, and higher medical costs. A study [26] aimed to measure the effects of surgical site infections. Found that infected patients had their hospital stay doubled, and readmission rates increased fivefold. Orthopedic patients are at risk for surface and deep infections due to the bacteria identified in the AL Nassiriyah research, which can significantly hinder their recovery and overall health outcomes.

Comparing these results with other studies, it is evident that the contamination rates and types of pathogens identified are consistent with findings from similar investigations in orthopedic and other surgical settings. For example, a study conducted in a tertiary care hospital reported that the most common contaminants in operating theaters were S. aureus and *P. aeruginosa*, with contamination rates ranging from 2% to 5% [27], similar to the 3.09% contamination rate observed in this study.

Another study from a hospital in Nigeria found that *Escherichia coli* and *Enterobacter spp*. were among the most frequently isolated bacteria in operating rooms [28], supporting the present study's findings. These comparisons suggest that the types of bacteria identified in the Imam Hussein Hospital's orthopedic theaters are typical of those found in surgical environments globally, underscoring the universal challenge of maintaining sterility in these settings.

The bacterial highest resistance was detected against amoxicillin and ampicillin, flowed by Cefaclor, Cefuroxime, Cefadroxil, and Erythromycin. Amoxicillin resistance pattern were decreased upon adding clavulate to amoxicillin. S.aureus demonstrated the highest resistance to Ampicillin and amoxicillin (5 isolates), followed by Amoxicillin/ Clavulate and Erythromycin (3 isolates), Cefaclor (2 isolates), only one isolate was resistant to Cefadroxil, Ceftazidime, Cefotaxim, Cefixim, and Gentamycin, While all the isolates were susceptible to the rest of the tested antibiotics. The highest resistance pattern was detected in *P. aeruginosa* isolates followed by *E. coli,* it shoed different resistance patterns to 14 antibiotics showing susceptibility to Amikacin only. The lowest resistant patterns were detected among Coagulase-negative *Staphylococci, Enterobacter spp*. & *Bacillus spp*. Which showed resistance to (4, 5, 4) antibiotics, respectively. Evaluation of the pathogens' antibiogram and contamination rate typically acts as an early warning indicator for timely intervention. Substantial resistance to amoxicillin, and ampicillin was found in this investigation; this trend was consistent with research done in underdeveloped nations [29].

However, in a study conducted by Mohammed et al., isolates showed resistance to two additional medications: Erythomycin & Gentamycin. These should raise concerns because they are among the most widely used and least expensive antibiotics. Antibiotic use may be the cause of variations in resistance patterns, which emphasizes the necessity of monitoring antimicrobial programs[30].

The temporal distribution of positive cultures, which showed higher contamination rates in February (28.57%), March (38.10%), and April (23.81%), with a decrease in May (9.52%), may be indicative of seasonal variations in microbial load or fluctuations in operating room usage and staff activity levels. This pattern could reflect the increased patient load and surgical procedures during the earlier months, potentially leading to higher contamination risks. Alternatively, it may suggest lapses in cleaning protocols during busier periods. The drop in contamination in May could be attributed to improved

cleaning practices, reduced surgical activity, or changes in environmental conditions. These findings highlight the need for continuous monitoring and adjustment of infection control measures throughout the year to address potential seasonal variations in contamination risk.

The study's findings significantly affect infection control practices in orthopedic operating rooms. Identifying specific contamination hotspots, such as the couch, floor, and walls, provides valuable information for targeted interventions to reduce microbial load in these areas. Regular and thorough cleaning of these surfaces and using effective disinfectants are essential to minimize the risk of patient exposure to harmful pathogens. Additionally, the study underscores the importance of maintaining strict sterility of surgical instruments and other critical items used in the operating room, as evidenced by the absence of bacterial growth on gauze and instruments. The results also suggest that ongoing staff education and training in infection control practices, particularly in the handling and cleaning high-risk areas, are crucial to sustaining low contamination rates.

CONCLUSIONS

The microbiological analysis of orthopedic operating theaters in Imam Hussein Hospital reveals a generally low but significant level of bacterial contamination, with specific pathogens posing a risk to patient safety. The study's findings are consistent with similar worldwide investigations, highlighting the universal challenges of maintaining sterile environments in surgical settings. Identifying contamination hotspots provides a basis for targeted interventions. At the same time, the temporal distribution of positive cultures suggests the need for continuous monitoring and adjustment of infection control measures. Ultimately, the study emphasizes the critical importance of rigorous infection control practices in preventing hospital-acquired infections and ensuring the safety of patients undergoing orthopedic surgery.

References

1. Nimer NA. Nosocomial infection and antibiotic-resistant threat in the Middle East. Infection and drug resistance. 2022; p. 631–9.
2. Menakaya C, et al. Consenting patients for elective procedures during the pandemic: Are we consenting for risk of nosocomial COVID-19 infection. Journal of Perioperative Practice. 2022; 32 (10): 270–4.
3. Huang W. What Do We Need to Know to Prevent and Control Nosocomial Infections Completely?-Part 2. Acta Scientific MICROBIOLOGY (ISSN: 2581-3226). 2022; 5 (4).
4. Chauveaux D. Preventing surgical-site infections: measures other than antibiotics. Orthopaedics & Traumatology: Surgery & Research. 2015; 101 (1): S77–S83.
5. Li K, et al., Effectiveness of preoperative antibiotics in preventing surgical site infection after common soft tissue procedures of the hand. Clinical Orthopaedics and Related Research. 2018; 476 (4): 664–73.
6. Yousufuddin S, et al., Surgical Site Infection in Hip Fracture Surgery; Reducing the Incidence of Infection and Correlation With co Morbidities and Blood Parameters. Int J Orthop. 2018; 5: 891–5.
7. Stambough JB, et al. Decreased hospital costs and surgical site infection incidence with a universal decolonization protocol in primary total joint arthroplasty. The Journal of arthroplasty. 2017; 32 (3): 728–34.
8. McQueen RH, Ehnes BL. Antimicrobial Textiles and Infection Prevention — Clothes and Inanimate Environment, in Infection Prevention: New Perspectives and Controversies. Springer, 2022; p. 139–49.
9. Owen L, Laird K. The role of textiles as fomites in the healthcare environment: a review of the infection control risk. PeerJ. 2020; 8: e9790.
10. Andersen BM, Andersen BM. Operation Department: Infection Control. Prevention and Control of Infections in Hospitals: Practice and Theory. 2019; p. 453–89.
11. Swenson D. Review of current practice in preventing health care associated infections, in Assurance of Sterility for Sensitive Combination Products and Materials. Elsevier, 2020; p. 135–64.
12. Bali RK. Operating room protocols and infection control. Oral and maxillofacial surgery for the clinician. 2021; p. 173–94.
13. Dallolio L, et al. Surveillance of environmental and procedural measures of infection control in the operating theatre setting. International journal of environmental research and public health. 2018; 15 (1): 46.
14. Graveto JM, Costa PJ, Santos CI. Cell phone usage by health personnel: preventive strategies to decrease risk of cross infection in clinical context. Texto & Contexto-Enfermagem. 2018; 27: e5140016.
15. Chaman R, et al. Survey of prevalence and types of bacterial contamination of mobile phones of personnel employed in major wards of educational hospitals in Yasuj. Journal of Fundamental and Applied Sciences. 2018; 10 (2).
16. Dancer SJ. Hospital cleaning: past, present, and future. Antimicrobial Resistance & Infection Control. 2023; 12 (1): 80.
17. Schinas G, et al. Preventing multidrug-resistant bacterial transmission in the intensive care unit with a comprehensive approach: a policymaking manual. Antibiotics. 2023; 12 (8): 1255.
18. Garba R, et al. Microbial pattern in urine samples of post repaired vesico-vaginal fistula patients at Laure Fistula Center, Kano, Nigeria. African Journal of Medicine and Medical Sciences. 2022; 51 (4): 275–85.
19. Shukla A, et al. Surveillance of microbiological environment of operation theaters. Cureus. 2021; 13 (12).
20. Infection prevention and control in-service education and training curriculum. World Health Organization, 2024.
21. Odoom A, Tetteh-Quarcoo PB, Donkor ES. Prevalence of Hospital-Acquired Infections in Low-and Middle-Income Countries: Systematic Review and Meta-Analysis. Asia Pacific Journal of Public Health. 2024; p. 10105395251338002.
22. Nedopil AJ, Howell SM. Operating Room Methods to Reduce Infection in Total Knee Arthroplasty. Infection in Knee Replacement. 2022; p. 339–46.
23. Parvizi J, et al. Environment of care: Is it time to reassess microbial contamination of the operating room air as a risk factor for surgical site infection in total joint arthroplasty? American journal of infection control. 2017; 45 (11): 1267–72.
24. Mwita JC, et al. Key issues surrounding appropriate antibiotic use for prevention of surgical site infections in low-and middle-income countries: a narrative review and the implications. International journal of general medicine. 2021; 515–30.
25. Behera B, et al. Impact of modified CDC/NHSN surveillance definition on the incidence of CAUTI: a study from an Indian tertiary care hospital. Journal of Infection Prevention. 2021; 22 (4): 162–5.
26. Totty JP, et al. The impact of surgical site infection on hospitalisation, treatment costs, and health–related quality of life after vascular surgery. International Wound Journal. 2021; 18 (3): 261–8.
27. Okey-Kalu EU, et al. Antibiogram and Plasmid Profile of Bacterial Isolates from Intensive Care Units in a Tertiary Healthcare Facility in Umuahia. Journal of Human Environment and Health Promotion. 2022; 8 (1): 10–14.
28. Abubakar S, et al. Spectrum of bacterial isolates among intensive care units patients in a tertiary hospital in northeastern Nigeria. Indian J Sci Resour Technol. 2014; 2 (6): 42–7.
29. Ramírez NS, et al. Identification, resistance, and susceptibility of microorganisms on healthcare workers' hands: a systematic review and meta-analysis. Revista de Epidemiologia e Controle de

Infecção. 2024; 14 (3): 1–13.
30. Mohammed A, et al. Bacterial contamination of operating theaters at a tertiary hospital in Bauchi, Northatsern Nigeria. EJPMR. 2017; 4 (4): 182–8.

Литература

1. Nimer NA. Nosocomial infection and antibiotic-resistant threat in the Middle East. Infection and drug resistance. 2022; p. 631–9.
2. Menakaya C, et al. Consenting patients for elective procedures during the pandemic: Are we consenting for risk of nosocomial COVID-19 infection. Journal of Perioperative Practice. 2022; 32 (10): 270–4.
3. Huang W. What Do We Need to Know to Prevent and Control Nosocomial Infections Completely?-Part 2. Acta Scientific MICROBIOLOGY (ISSN: 2581-3226). 2022; 5 (4).
4. Chauveaux D. Preventing surgical-site infections: measures other than antibiotics. Orthopaedics & Traumatology: Surgery & Research. 2015; 101 (1): S77–S83.
5. Li K, et al., Effectiveness of preoperative antibiotics in preventing surgical site infection after common soft tissue procedures of the hand. Clinical Orthopaedics and Related Research. 2018; 476 (4): 664–73.
6. Yousufuddin S, et al., Surgical Site Infection in Hip Fracture Surgery; Reducing the Incidence of Infection and Correlation With co Morbidities and Blood Parameters. Int J Orthop. 2018; 5: 891–5.
7. Stambough JB, et al. Decreased hospital costs and surgical site infection incidence with a universal decolonization protocol in primary total joint arthroplasty. The Journal of arthroplasty. 2017; 32 (3): 728–34.
8. McQueen RH, Ehnes BL. Antimicrobial Textiles and Infection Prevention — Clothes and Inanimate Environment, in Infection Prevention: New Perspectives and Controversies. Springer, 2022; p. 139–49.
9. Owen L, Laird K. The role of textiles as fomites in the healthcare environment: a review of the infection control risk. PeerJ. 2020; 8: e9790.
10. Andersen BM, Andersen BM. Operation Department: Infection Control. Prevention and Control of Infections in Hospitals: Practice and Theory. 2019; p. 453–89.
11. Swenson D. Review of current practice in preventing health care associated infections, in Assurance of Sterility for Sensitive Combination Products and Materials. Elsevier, 2020; p. 135–64.
12. Bali RK. Operating room protocols and infection control. Oral and maxillofacial surgery for the clinician. 2021; p. 173–94.
13. Dallolio L, et al. Surveillance of environmental and procedural measures of infection control in the operating theatre setting. International journal of environmental research and public health. 2018; 15 (1): 46.
14. Graveto JM, Costa PJ, Santos CI. Cell phone usage by health personnel: preventive strategies to decrease risk of cross infection in clinical context. Texto & Contexto-Enfermagem. 2018; 27: e5140016.
15. Chaman R, et al. Survey of prevalence and types of bacterial contamination of mobile phones of personnel employed in major wards of educational hospitals in Yasuj. Journal of Fundamental and Applied Sciences. 2018; 10 (2).
16. Dancer SJ. Hospital cleaning: past, present, and future. Antimicrobial Resistance & Infection Control. 2023; 12 (1): 80.
17. Schinas G, et al. Preventing multidrug-resistant bacterial transmission in the intensive care unit with a comprehensive approach: a policymaking manual. Antibiotics. 2023; 12 (8): 1255.
18. Garba R, et al. Microbial pattern in urine samples of post repaired vesico-vaginal fistula patients at Laure Fistula Center, Kano, Nigeria. African Journal of Medicine and Medical Sciences. 2022; 51 (4): 275–85.
19. Shukla A, et al. Surveillance of microbiological environment of operation theaters. Cureus. 2021; 13 (12).
20. Infection prevention and control in-service education and training curriculum. World Health Organization, 2024.
21. Odoom A, Tetteh-Quarcoo PB, Donkor ES. Prevalence of Hospital-Acquired Infections in Low-and Middle-Income Countries: Systematic Review and Meta-Analysis. Asia Pacific Journal of Public Health. 2024; p. 10105395251338002.
22. Nedopil AJ, Howell SM. Operating Room Methods to Reduce Infection in Total Knee Arthroplasty. Infection in Knee Replacement. 2022; p. 339–46.
23. Parvizi J, et al. Environment of care: Is it time to reassess microbial contamination of the operating room air as a risk factor for surgical site infection in total joint arthroplasty? American journal of infection control. 2017; 45 (11): 1267–72.
24. Mwita JC, et al. Key issues surrounding appropriate antibiotic use for prevention of surgical site infections in low-and middle-income countries: a narrative review and the implications. International journal of general medicine. 2021; 515–30.
25. Behera B, et al. Impact of modified CDC/NHSN surveillance definition on the incidence of CAUTI: a study from an Indian tertiary care hospital. Journal of Infection Prevention. 2021; 22 (4): 162–5.
26. Totty JP, et al. The impact of surgical site infection on hospitalisation, treatment costs, and health–related quality of life after vascular surgery. International Wound Journal. 2021; 18 (3): 261–8.
27. Okey-Kalu EU, et al. Antibiogram and Plasmid Profile of Bacterial Isolates from Intensive Care Units in a Tertiary Healthcare Facility in Umuahia. Journal of Human Environment and Health Promotion. 2022; 8 (1): 10–14.
28. Abubakar S, et al. Spectrum of bacterial isolates among intensive care units patients in a tertiary hospital in northeastern Nigeria. Indian J Sci Resour Technol. 2014; 2 (6): 42–7.
29. Ramírez NS, et al. Identification, resistance, and susceptibility of microorganisms on healthcare workers' hands: a systematic review and meta-analysis. Revista de Epidemiologia e Controle de Infecção. 2024; 14 (3): 1–13.
30. Mohammed A, et al. Bacterial contamination of operating theaters at a tertiary hospital in Bauchi, Northatsern Nigeria. EJPMR. 2017; 4 (4): 182–8.

CD4/CD8 RATIO AS A HIV-ASSOCIATED FACTOR OF THE LUNG CANCER COURSE AND OUTCOME PROGNOSIS

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Non-AIDS-defining cancers represent one of the leading causes of death among people living with HIV in developed economies due to successful antiretroviral therapy. Malignant neoplasms (MNs) of the lung occupy leading positions in prevalence and mortality, affecting younger people compared to the general population. Despite the fact that the role of HIV in the direct mechanism underlying the lung cancer carcinogenesis has not been proven, the immunodeficiency-mediated effect, including that on the anti-tumor immunity, contributes to the earlier neoplastic process development and to the features of the disease course and anti-tumor treatment. HIV often becomes an exclusion criterion for multiple oncology clinical trials, and this group of patients is overlooked. The study aimed to assess the impact of the CD4/CD8 ratio as one of the key markers of the state of cell-mediated immunity on the lung cancer course prognosis during anti-tumor treatment. The data of 17 HIV patients with MNs of the lung and 31 non-HIV patients of the control group, who underwent treatment in 2018–2023, were analyzed. The analysis determined the threshold CD4/CD8 ratio value (≤ 0.57) and the fact of its decrease by more than 0.01, which reflected a significant overall survival worsening ($p < 0.05$) during lung cancer treatment. Furthermore, comparative analysis of patients of the index and control groups revealed no significant differences in progression-free survival and the number of therapy lines, which suggests comparable treatment outcomes in patients with lung cancer against the background of existing HIV ($p > 0.05$).

Keywords: lung cancer, HIV, non-AIDS-defining cancers, CD4/CD8 ratio

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ИММУНОРЕГУЛЯТОРНЫЙ ИНДЕКС КАК ВИЧ-АССОЦИИРОВАННЫЙ ФАКТОР ПРОГНОЗА ТЕЧЕНИЯ И ИСХОДА РАКА ЛЕГКОГО

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В развитых странах благодаря успешной антиретровирусной терапии ВИЧ-неассоциированные опухоли являются одной из ведущих причин летальных исходов среди людей, живущих с ВИЧ. Злокачественные новообразования (ЗНО) легких занимают лидирующие позиции по частоте встречаемости и смертности, затрагивая лиц более молодого возраста по сравнению с общей популяцией. Несмотря на то что роль ВИЧ-инфекции в прямом механизме онкогенеза рака легкого не доказана, опосредованное через иммунодефицит влияние в том числе и на противоопухолевый иммунитет способствует более раннему развитию опухолевого процесса, вносит особенности в течение заболевания и в противоопухолевое лечение. Зачастую ВИЧ-инфекция является критерием исключения из большего числа клинических исследований в онкологии, и данная группа пациентов остается без внимания. Целью исследования было изучить влияние индекса CD4/CD8 как одного из ключевых маркеров состояния клеточного иммунитета на прогноз течения рака легкого в ходе противоопухолевого лечения. Анализировали данные 17 пациентов с ЗНО легких и ВИЧ-инфекцией и данные 31 пациента контрольной группы без ВИЧ, проходивших лечение с 2018 по 2023 г. В ходе анализа определены пороговый уровень индекса CD4/CD8, составивший ≤ 0,57, и его динамика снижения более чем на 0,01, которые значимо отражали ухудшение общей выживаемости ($p < 0,05$) в ходе лечения рака легкого. Кроме того, в ходе сравнительного анализа основной и контрольной групп пациентов не было выявлено статических значимых различий в выживаемости без прогрессирования и в числе линий терапии, что позволяет сделать предположение о сопоставимых результатах лечения больных раком легкого на фоне имеющейся ВИЧ-инфекции ($p > 0,05$).

Ключевые слова: рак легкого, ВИЧ-инфекция, ВИЧ-неассоцированные ЗНО, иммунорегуляторный индекс

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There were major changes in the structure of mortality and the increase in life expectancy among people with HIV due to the state strategy of countering the spread of HIV in the Russian Federation (RF), extensive coverage and availability of advanced antiretroviral therapy (ART). The share of malignant neoplasms (MNs) in the structure of morbidity and mortality of this population increases with increasing overall survival and age of HIV-infected people. Today, in industrialized countries, non-AIDS-defining cancers result in the larger number of deaths, than AIDS-defining ones, hepatitis C, or cardiovascular disorders. It is the second (after AIDS) most common cause of death among HIV-infected patients [1, 2].

In the RF and Russian literature, it is common to allocate AIDS-defining cancers (ADCs) that include Kaposi's sarcoma, primary central nervous system lymphoma, non-Hodgkin lymphomas, invasive cervical cancer. Detection of those suggests AIDS stage in a HIV patient. Furthermore, non-AIDS-defining cancers (NADCs) are distinguished that are most common in early-stage HIV (hepatocellular carcinoma, lung cancer, squamous cell carcinoma of the head and neck, anal canal cancer, Hodgkin lymphoma) [3–5].

The most common NADCs were reported in one of the largest studies of HIV-infected population in the USA, Europe, and Australia D:A:D 3 (176,775 HIV patients, among them 880 cases of NADCs in 2004–2010): lung cancer (0.79/1000 people/year), Hodgkin lymphoma (0.63/1000 people/year), and anal canal cancer (0.45/1000 people/year) [6]. The nationwide Japanese study conducted in 2024 (Longitudinal Annual Survey of HIV/AIDS Referral Hospitals in Japan From 1999 to 2021: Trend in Non-AIDS-defining Cancers Among Individuals Infected With HIV-1) showed an upward trend of NADC incidence in 1999–2021, and lung cancer was the most prevalent MN form (14%) [7].

In HIV-infected patients, lung cancer is diagnosed on average ten years earlier. Even in the group of nonsmokers, HIV was associated with the 4-fold increased risk of death from lung cancer relative to the general population. According to the data of the same Japanese study, lung cancer was second only to pancreatic cancer in mortality rate [8].

Despite the decrease in the rate of HIV incidence from 43.29 to 40.04 per 100,000 population in 2023 (by 7.5% compared to the year 2022), the number of new cases exceeds the number of deaths, and the total number of people living with HIV in Russia is still growing [9]. The relevance of treatment of this group of patients is growing every year. However, HIV is an exclusion criterion for the majority of multicenter randomized clinical trials of cancer treatment. And, therefore, the prevalence and nature of cancer in HIV-infected patients are poorly understood, including lung cancer, its clinical and morphological features against the background of HIV-mediated factors. The prognosis, that was extremely bad in the era when there was no antiretroviral therapy, can currently be almost the same as the prognosis of people having no HIV with the multidisciplinary approach, patient's adherence to treatment, and timely prevention.

The study published in the Journal of Thoracic Oncology in July 2025 involved comparative analysis of immunogenomic characteristics of non-small-cell lung cancer (NSCLC) in people living with HIV (PLWHIV) and immunocompetent patients. HIV severely impairs both systemic and local antitumor immunity, despite similar molecular profiles of the tumor, through severe persistent impairment of the interaction between the CD4 and CD8 T cells. This can contribute to worse prognosis of NSCLC in PLWHIV, even in cases of virological control [10]. One HIV-mediated factor is the CD4/CD8 ratio that has shown its significance as a biomarker for lung cancer screening and a factor of poor prognosis in a number of studies [11–13].

Thus, the study aimed to assess the impact of HIV-associated immunological factors on the lung cancer course prognosis during antitumor treatment, as well as to analyze progression-free survival relative to the control group.

METHODS

Inclusion criteria: malignant neoplasm of the lung; registered diagnosis of HIV infection. Exclusion criteria: patient's blood levels of CD4 lymphocytes < 200 cells/mL; exacerbation of chronic infectious disease.

We conducted a prospective study of diagnostic value of the CD4/CD8 ratio as a HIV-mediated predictor of fatal lung cancer outcome. The index group included 17 patients with stage I–IV lung cancer and HIV infection, the control group included 31 individuals with lung cancer having no HIV, who had been treated in 2018–2023 at the St. Petersburg City Clinical Oncology Dispensary.

All 17 patients included in the study underwent anti-retroviral therapy in accordance with the current clinical guidelines on NIV treatment issued in appropriate year with assessment of viral load and cell-mediated immunity state after each even-numbered drug therapy cycle or a month after surgical treatment. Together with the infectious disease physician we assessed the need to adjust antiretroviral (ARV) drugs in order to reduce overlapping toxicity in accordance with antitumor drug treatment regimen. The patients included in the index and control groups were comparable based on the main clinical and demographic characteristics (Tables 1, 2).

R ver. 4.1.1 statistical environment (R Core Team, 2020) [14] and MedCalc (ver. 23.1.1) software were used for statistical analysis.

Significance level was set as $p < 0.05$. *P*-values are presented in the report with two decimal places, if exceed 0.05, and with three decimal places at $p < 0.05$.

The qualitative characteristic description is provided as absolute number of observations (*n*), percentage (%), and 95% confidence interval (CI) for shares.

Survival analysis was performed using the Kaplan–Meier estimator; comparison of survival curves between patients in the groups was performed using the log-rank test.

Sensitivity and specificity of significant models were tested using ROC curves. The cut-off value for the model was determined by maximization of the sum of sensitivity and specificity or by determining the sensitivity and specificity balance and the Youden's index.

Based on the predictor analysis results in univariate models, all the predictors identified were integrated into a multivariate model for analysis of the relationship between indicators combined with the fatal outcome probability. The multivariate logistic regression model was considered significant at $p < 0.05$ for all independent traits.

RESULTS

The CD4/CD8 ratio varied between 0.13 and 1.11 in patients of the index group, the median value was 0.79.

ROC analysis was used to assess the impact of baseline CD4/CD8 ratio values and dynamic CD4/CD8 changes during antitumor treatment on the overall survival (OS) of patients of the index group. Optimal threshold values of the indicators considered were determined. The univariate analysis conducted revealed a significant impact of the CD4/CD8 ratio

Table 1. Characteristics of the group of HIV patients and the control group

Groups based on the age of cancer detection (WHO classification)		
Group	Parameter	Rate (share)
HIV patients	Young age (18–44 years)	5/17 (29.5%)
	Middle age (45–59 years)	8/17 (47%)
	Advanced age (60–74 years)	4/17 (23.5%)
Non-HIV patients	Young age (18–44 years)	0/32 (0%)
	Middle age (45–59 years)	15/32 (46.9%)
	Advanced age (60–74 years)	17/32 (53.1%)
Patient distribution by gender		
Group	Parameter	Rate (share)
HIV patients	Male	12/17 (70.6%)
	Female	5/17 (29.4%)
Non-HIV patients	Male	19/32 (61.3%)
	Female	12/32 (38.7%)
Stage I–IV cancer		
Group	Parameter	Rate (share)
HIV patients	IA	2/17 (11.8%)
	IB	0/17 (0%)
	IIA	1/17 (5.9%)
	IIB	2/17 (11.8%)
	IIIA	2/17 (11.8%)
	IIIB	1/17 (5.9%)
	IV	7/17 (41.2%)
	IVA	1/17 (5.9%)
	IVB	1/17 (5.9%)
Non-HIV patients	IA	4/32 (12.9%)
	IB	1/32 (3.2%)
	IIA	0/32 (0%)
	IIB	3/32 (9.7%)
	IIIA	6/32 (19.4%)
	IIIB	7/32 (22.6%) 10.3–41.5%
	IV	7/32 (22.6%)
	IVA	3/32 (9.7%)
	IVB	0/32 (0%)
Histologic type: Adenocarcinoma among HIV and non-HIV patients		
Group	Rate (share)	
HIV patients	7/17 (41.2%)	
Non-HIV patients	19/31 (61.3%)	
Histologic type: Squamous cell carcinoma among HIV and non-HIV patients		
Group	Частота (доля)	
HIV patients	5/17 (29.4%)	
Non-HIV patients	8/31 (25.8%)	
HIV patients	4/17 (23.5%)	
Non-HIV patients	3/31 (9.7%)	
Histologic type: Atypical carcinoid among HIV and non-HIV patients		
Group	Rate (share)	
HIV patients	1/17 (5.9%)	
Non-HIV patients	0/31 (0%)	
Patient distribution by gender		
Parameter	Rate (share)	
Male	12/17 (70.6%) 44–88.6%	
Female	5/17 (29.4%) 11.4–56%	

Table 2. Characteristics of the group of HIV patients

HIV infection stages	
Parameter	Rate (share)
3	1/17 (5.9%)
3 remission	1/17 (5.9%)
4A remission	3/17 (17.6%)
4B remission	5/17 (29.4%)
4C progression	5/17 (29.4%)
4C remission	2/17 (11.8%)
Viral load at the time of cancer detection (copies/mL)	
N	17
M	35106.41
SD	89540.49
95% CI	–7457.65; 77670.47
Min	20
Max	364467
Me	1052
IQR	40; 14696
CD4 cells at the time of cancer detection (cells/mL)	
N	17
M	370.94
SD	120.99
95% CI	313.43; 428.45
Min	252
Max	631
Me	317
IQR	277; 445
CD4/CD8 index at the time of cancer detection	
N	17
M	0.7
SD	0.31
95% CI	0.55; 0.85
Min	0.13
Max	1.11
Me	0.79
IQR	0.48; 0.92

on the overall survival of patients with MNs of the lung and concomitant HIV infection ($p < 0.005$) (Table 3, Fig. 1, 2).

Multivariate analysis was performed using the Cox proportional hazards model, which included the CD4/CD8 ratio and the CD4/CD8 ratio dynamic changes during antitumor treatment that had an effect on survival when applying univariate ROC analysis. The multivariate analysis results showed the following: the baseline CD4/CD8 ratio value (before treatment) and the CD4/CD8 ratio dynamic changes during the ongoing therapy turned out to be independent prognostic factors having a significant effect on the OS of patients with MNs of the lung and HIV (overall significance of the model: $p < 0.0001$, AUC = 0.920 (95% CI 0.851– 0.989)) (Table 4).

With the threshold CD4/CD8 ratio value of 0.57, the median OS of the patients with the ratio values above the threshold value at the time of data set acquisition had not been achieved and was significantly different from the median OS of the patients with the threshold or lower CD4/CD8 ratio values, in whom it was 3 months ($p = 0.0117$) (Fig. 3).

The median OS of the patients, in whom the CD4/CD8 ratio decreased by more than 0.01, was 4 months. It was significantly lower, than that of the patients, in whom the CD4/CD8 ratio did not decrease to the threshold level ($p = 0.0220$) (Fig. 4).

Furthermore, prediction of fatal outcomes revealed a positive correlation with male sex in HIV-infected patients with lung cancer. The likelihood of fatal outcome in males is 5.27 times higher, than in females ($p < 0.05$) (Fig. 5).

Lung cancer in HIV-infected patients of the index group was treated in accordance with the clinical guidelines of appropriate year. Five patients (16.1%) underwent surgical treatment, 14 (45.2%) received combination treatment, eight patients (47.2%) received drug therapy in the therapeutic regimen. Sixteen patients out of 17 were prescribed antitumor drug treatment, and only one patient (5.8%) with small-cell lung cancer died before the beginning of chemotherapy. In this sample of patients, radiotherapy was not prescribed as a method to treat the locally advanced form of lung cancer.

Comparative analysis revealed no significant differences in the number of therapy lines and progression-free survival between HIV and non-HIV patients (Table 5).

Table 3. Results of univariate analysis of the impact of clinical-morphological and immunological factors considered on the OS of HIV patients

Indicator	Baseline CD4/CD8	CD4/CD8 dynamic changes during treatment
Optimal threshold (cut-off) value	≤ 0.57	Decrease by no more than 0.01 from baseline

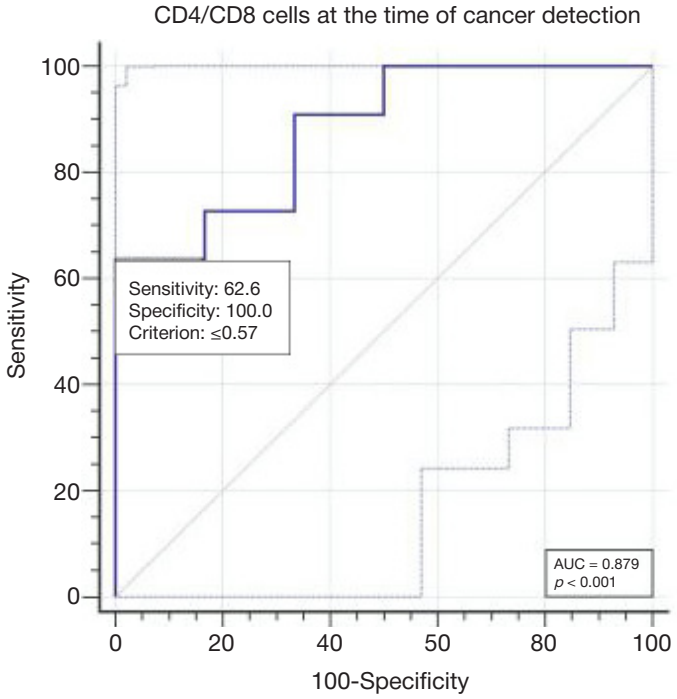

Fig. 1. Results of ROC analysis aimed at determining threshold CD4/CD8 ratio (Youden's index) values at the time of lung cancer detection in HIV-infected patients

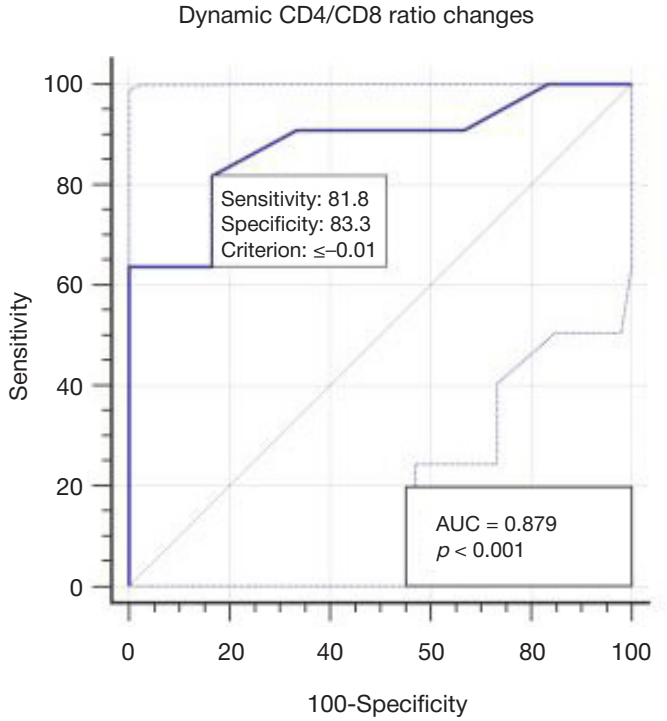

Fig. 2. Results of ROC analysis aimed at determining the dynamic changes in threshold CD4/CD8 ratio (Youden's index) values during antitumor treatment in HIV-infected patients

Table 4. Results of miltivariate analysis of the impact of clinical-morphological and immunological factors considered on the OS of HIV patients ($p < 0.0001$, AUC = 0.920 (95% CI 0.851–0.989))

Indicators included in the model based on the univariate analysis results	OS (95% CI)	*p*-value
Baseline CD4/CD8	0.0006 (0.00–0.19)	0.0117
CD4/CD8 dynamic changes during treatment (relative to baseline)	0.0001 (0.00–0.27)	0.022

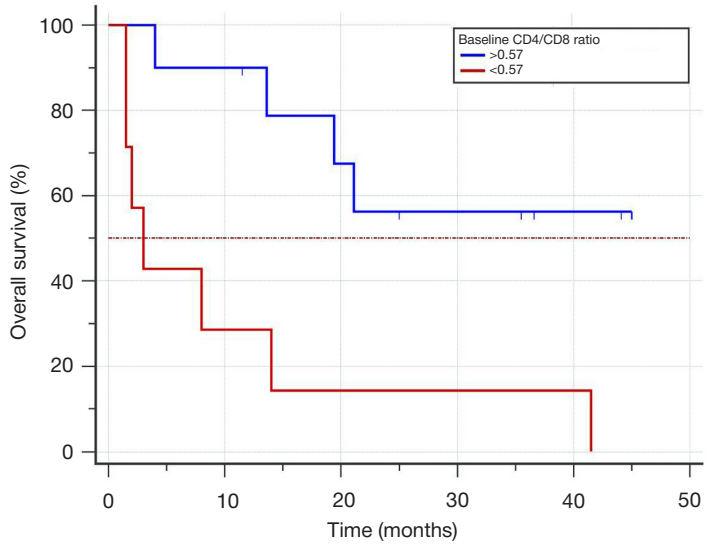

Fig. 3. Miltivariate analysis results: overall survival as a function of baseline CD4/CD8 ratio in HIV-infected patients

DISCUSSION

Low CD4/CD8 ratio value reflects disorganization and dysfunction of the immune system, including antitumor immunity; it is correlated to the degree of lung cancer dissemination and, therefore, indirectly to the neoplastic process severity [15], as well as to potential antitumor therapy efficacy. The threshold CD4/CD8 ratio value of 0.57 and below, as well as the CD4/CD8 ratio decrease by 0.01 during treatment as a HIV-mediated predictor of fatal outcome significantly demonstrates poor prognosis in patients with lung cancer and concomitant HIV infection, which can be used in clinical practice ($p < 0.05$).

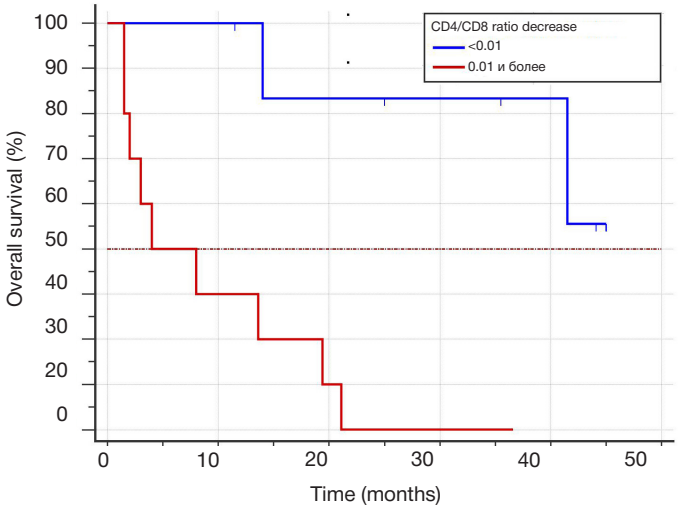

Fig. 4. Miltivariate analysis results: overall survival as a function of the dynamic CD4/CD8 ratio changes during antitumor treatment in HIV-infected patients

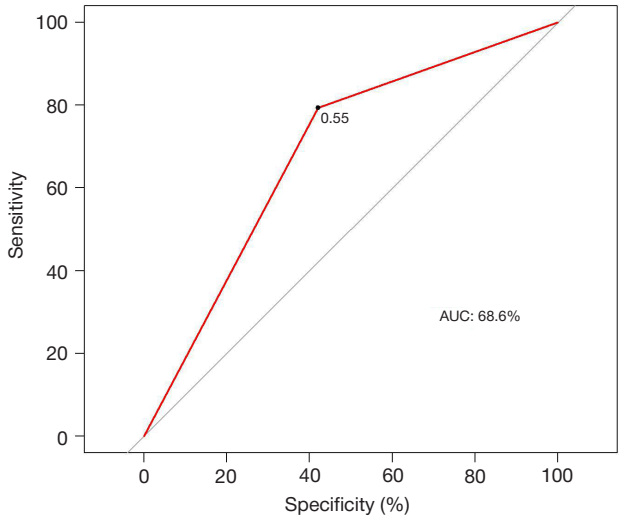

Fig. 5. Fatal outcome likelihood as a function of the “male sex” trait in HIV-infected patients

Table 5. Progression-free survival by therapy lines in the index group of HIV-infected patients and the control group

Group	Parameter	Number of therapy lines	PFS 1st line therapy, months	PFS 2nd line therapy, months	PFS 3rd line therapy, months	PFS 4th line therapy, months
HIV patients	N	17	16	6	2	1
	M	1.47	15.24	7.43	2.99	0.54
	SD	0.94	16.49	5.64	3.75	–
	95% CI	1.02; 1.92	7.4; 23.08	4.75; 10.11	1.21; 4.77	–
	Min	0	0.92	1.61	0.34	0.54
	Max	4	44.75	14.17	5.64	0.54
	Me	1	9.3	6.47	2.99	0.54
	IQR	1; 2	1.52; 28.9	2.64; 12.48	1.67; 4.31	0.54; 0.54
Non-HIV patients	N	31	31	16	7	2
	M	1.81	13.96	6.98	4.86	1.83
	Me	2	9.61	6.51	3.58	1.83
	IQR	1; 2	5.37; 24.78	3.16; 8.71	2.76; 6.8	1.41; 2.26
Test		Mann–Whitney test	Mann–Whitney test	Student's *t*-test	Mann–Whitney test	Mann–Whitney test
p-value for intergroup differences		0.21	0.7	0.87	0.67	0.67

Note: standard deviation and 95% CI for the average time to progression during 4th line therapy in the group of HIV patients could not be calculated, since this value was available for only one patient in this group (marked with "–").

The analysis of antitumor treatment efficacy relative to the control group of non-HIV patients revealed no significant differences in the number of therapy lines and progression-free survival between patients, which showed current options for simultaneous lung cancer treatment and HIV infection control with the multidisciplinary approach involving regular evaluation of the cell-mediated immunity indicators.

The fact of the relationship between high mortality rate and male sex suggests the direct effect of HIV on the lung cancer carcinogenesis, along with smoking, since people living with HIV, both males and females, are historically more likely to smoke tobacco.

CONCLUSIONS

When predicting probability of the lung cancer fatal outcome based the HIV-associated predictors, the baseline CD4/CD8 ratio value and the dynamic CD4/CD8 ratio changes during antitumor treatment had a significant effect on the OS ($p < 0.05$). Identification of such predictors makes it possible to determine the group at high risk of death from lung cancer among HIV-infected patients, requiring more intense therapy and additional discussion of treatment tactics for the patient with the infectious disease physician for potential ART adjustment. Such stratification can either be an extra argument not to reduce the dose of chemotherapy drugs against the background of hematological and other toxic complications or in patients with borderline functional status, or provide the basis for the antiretroviral drug prescription revision. Regular laboratory assessment of the cell-mediated immunity state represents an inevitable part of the comprehensive approach to treatment of this group of patients. If algorithms for management of patients with concomitant HIV infection are developed and introduced into clinical practice, the results of antitumor treatment for lung cancer can be comparable with that in the general population.

References

1. Smith CJ, Ryom L, Weber R, et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. Lance.t 2014; 384: 241–8.
2. Trickey A, Sabin CA, Burkholder G, et al. Life expectancy after 2015 of adults with HIV on long-term antiretroviral therapy in Europe and North America: a collaborative analysis of cohort studies. Lancet HIV. 2023; 10 (5): 295–307.
3. Centers for Disease Control and Prevention. HIV Surveillance Report, 2016. Available from: vol.28http://www.cdc.gov/hiv/library/reports/hiv-surveillance.html. Published November 2017. Accessed June 2019.
4. Orenstein JM. Ultrastructure of HIV/AIDS. Ultrastruct Pathol. 2002; 26: 245–50.
5. Nekrasova AV. Kliniko-morfologicheskaia kharakteristika, rezul'taty lecheniia i prognoz zlokachestvennykh novoobrazovanii pri VICH-infektsii. Obninsk, 2021. Dostupno po ssylke: https://kgma.info/files/dissovet/diss_nav_22_12_2021.pdf.
6. Worm SW, Bower M, Reiss P, et al. Non-AIDS defining cancers in the D:A:D study-time trends and predictors of survival: a cohort study. BMC Infect Dis. 2013; 13: 471. DOI: 10.1186/1471-2334-13-471.
7. Tanaka T, Oshima K, Kawano K, et al. Nationwide Longitudinal Annual Survey of HIV/AIDS Referral Hospitals in Japan From 1999 to 2021: Trend in Non-AIDS-defining Cancers Among Individuals Infected With HIV-1. J Acquir Immune Defic Syndr. 2024; 96 (1): 1–10. DOI: 10.1097/QAI.0000000000003389.
8. Marcus JL, Chao C, Leyden WA, et al. Survival among HIV-infected and HIV-uninfected individuals with common non-AIDS-defining cancers. Cancer Epidemiol Biomarkers Prev. 2015; 24 (8): 1167–73. DOI: 10.1158/1055-9965.EPI-14-1079.
9. VICH-infektsiia v Rossiiskoi Federatsii na 31 dekabria 2022 g. Dannye polucheny v 1987–2023 gg. iz territorial'nykh tsentrov po profilaktike i bor'be so SPIDom (ili inykh upolnomochennykh organizatsii) i territorial'nykh Upravlenii Federal'noi sluzhby po nadzoru v sfere zashchity prav potrebitelei i blagopoluchiia cheloveka. Spetsializirovannyi nauchno-issledovatel'skii otdel po profilaktike i bor'be so SPIDom FBUN TSentral'nogo NII epidemiologii Rospotrebnadzora. Dostupno po ssylke: http://www.hivrussia.info/wp-content/uploads/2023/09/

Spravka-VICH-v-Rossii-na-31.12.2022.pdf.
10. Abbar B, Labreche K, Cadranel J, et al. Human Immunodeficiency Virus Impairs Immune Responses to Tumor Neoepitopes Without Altering Mutational Profiles in NSCLC. J Thorac Oncol. 2025; 20 (7): 897–911. DOI: 10.1016/j.jtho.2025.02.001. Epub 2025 Feb 7. PMID: 39923848.
11. Castilho JL, Bian A, Jenkins CA, et al North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of the International Epidemiology Databases to Evaluate AIDS (IeDEA). CD4/CD8 Ratio and Cancer Risk Among Adults With HIV. J Natl Cancer Inst. 2022; 114 (6): 854–62.
12. Clifford GM, Lise M, Franceschi S, et al CD4/CD8 ratio and lung cancer risk. Lancet HIV. 2017; 4 (3): 103.
13. Klugman M, Fazzari M, Xue X, et al. The associations of CD4 count, CD4/CD8 ratio, and HIV viral load with survival from non-small cell lung cancer in persons living with HIV. AIDS Care. 2022; 34 (8): 1014–21. DOI: 10.1080/09540121.2021.1934380. Epub 2021 Jun 1. PMID: 34074183; PMCID: PMC8633167.
14. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2020.
15. Manikhas GM, Zakharenko AA, Polezhaev DA, et al. Definition of the CD4/CD8 index as a possible criterion for the severity of the course and prognosis of lung cancer among HIV-infected people. Palliative medicine and rehabiliation. 2023; 1:15-19.

Литература

1. Smith CJ, Ryom L, Weber R, et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. Lance.t 2014; 384: 241–8.
2. Trickey A, Sabin CA, Burkholder G, et al. Life expectancy after 2015 of adults with HIV on long-term antiretroviral therapy in Europe and North America: a collaborative analysis of cohort studies. Lancet HIV. 2023; 10 (5): 295–307.
3. Centers for Disease Control and Prevention. HIV Surveillance Report, 2016. Available from: vol.28http://www.cdc.gov/hiv/library/reports/hiv-surveillance.html. Published November 2017. Accessed June 2019.
4. Orenstein JM. Ultrastructure of HIV/AIDS. Ultrastruct Pathol. 2002; 26: 245–50.
5. Некрасова А. В. Клинико-морфологическая характеристика, результаты лечения и прогноз злокачественных новообразований при ВИЧ-инфекции. Обнинск, 2021. Доступно по ссылке: https://kgma.info/files/dissovet/diss_nav_22_12_2021.pdf.
6. Worm SW, Bower M, Reiss P, et al. Non-AIDS defining cancers in the D:A:D study-time trends and predictors of survival: a cohort study. BMC Infect Dis. 2013; 13: 471. DOI: 10.1186/1471-2334-13-471.
7. Tanaka T, Oshima K, Kawano K, et al. Nationwide Longitudinal Annual Survey of HIV/AIDS Referral Hospitals in Japan From 1999 to 2021: Trend in Non-AIDS-defining Cancers Among Individuals Infected With HIV-1. J Acquir Immune Defic Syndr. 2024; 96 (1): 1–10. DOI: 10.1097/QAI.0000000000003389.
8. Marcus JL, Chao C, Leyden WA, et al. Survival among HIV-infected and HIV-uninfected individuals with common non-AIDS-defining cancers. Cancer Epidemiol Biomarkers Prev. 2015; 24 (8): 1167–73. DOI: 10.1158/1055-9965.EPI-14-1079.
9. ВИЧ-инфекция в Российской Федерации на 31 декабря 2022 г. Данные получены в 1987–2023 гг. из территориальных центров по профилактике и борьбе со СПИДом (или иных уполномоченных организаций) и территориальных Управлений Федеральной службы по надзору в сфере защиты прав потребителей и благополучия человека. Специализированный научно-исследовательский отдел по профилактике и борьбе со СПИДом ФБУН Центрального НИИ эпидемиологии Роспотребнадзора. Доступно по ссылке: http://www.hivrussia.info/wp-content/uploads/2023/09/Spravka-VICH-v-Rossii-na-31.12.2022.pdf.
10. Abbar B, Labreche K, Cadranel J, et al. Human Immunodeficiency Virus Impairs Immune Responses to Tumor Neoepitopes Without Altering Mutational Profiles in NSCLC. J Thorac Oncol. 2025; 20 (7): 897–911. DOI: 10.1016/j.jtho.2025.02.001. Epub 2025 Feb 7. PMID: 39923848.
11. Castilho JL, Bian A, Jenkins CA, et al North American AIDS Cohort Collaboration on Research and Design (NA-ACCORD) of the International Epidemiology Databases to Evaluate AIDS (IeDEA). CD4/CD8 Ratio and Cancer Risk Among Adults With HIV. J Natl Cancer Inst. 2022; 114 (6): 854–62.
12. Clifford GM, Lise M, Franceschi S, et al CD4/CD8 ratio and lung cancer risk. Lancet HIV. 2017; 4 (3): 103.
13. Klugman M, Fazzari M, Xue X, et al. The associations of CD4 count, CD4/CD8 ratio, and HIV viral load with survival from non-small cell lung cancer in persons living with HIV. AIDS Care. 2022; 34 (8): 1014–21. DOI: 10.1080/09540121.2021.1934380. Epub 2021 Jun 1. PMID: 34074183; PMCID: PMC8633167.
14. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. 2020.
15. Манихас Г. М., Захаренко А. А., Полежаев Д. А. и др. Определение CD4/CD8-индекса, как возможного критерия тяжести течения и прогноза рака легкого среди ВИЧ-инфицированных. Паллиативная медицина и реабилитация. 2023; 1: 15–19.

DETERMINATION OF AGING PHENOTYPE BASED ON THE CHANGES IN SPONTANEOUS AND INDUCED INTERLEUKIN-6 AND INTERLEUKIN-10 PRODUCTION *IN VITRO*

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During the aging the immune system alterations are accompanied by developing the systemic, sterile inflammation: inflammaging. Successful and pathological aging phenotypes are distinguished. Inflammaging severity depends largely on the ratio of pro- and anti-inflammatory mediators, especially IL6 and IL10. The study aimed to conduct the analysis of IL6 and IL10 production in the cultures of the patients' peripheral blood mononuclear cells (MNCs) as a possible approach to determining the aging phenotype. The data of elderly patients (n = 80), senile patients (n = 100), and centenarians (n = 30) were included in the study. Among those the groups were allocated with the successful and pathological phenotypes, along with the comparison group (young adults). The stimulation coefficient (SC) was assessed based on the ratio of the levels of stimulated and induced cytokine production. For the successful phenotype in elderly and senile individuals, as well as centenarians, a decrease in the IL6 SC to 5.3 [2.2–14.3] (p < 0.01), 5.3 [3.01–7.8] (p < 0.01), 6.5 [5.2–14.1], respectively, was reported, against the comparison group, where the value was 17.6 [13.7–31.1] (p < 0.05). With the pathological phenotype, the IL6 SC values of the studied age group showed no significant differences from that of the comparison group. For the successful phenotype in senile individuals, the increase in the IL10 SC to 6.9 [3.8–13.8] relative to the values of the group with the pathological phenotype — 3.3 [2.0–5.9] (p < 0.01) and the comparison group — 2.0 [1.9–2.2] (p < 0.001) was reported. In the group of centenarians with the pathological phenotype, there was a significant increase in the IL10 SC (11.2 [5.4–18.1] vs 2.7 [2.3–6.5] p < 0.001) in the group with successful aging, which can indicate the pronounced compensatory anti-inflammatory reserve being a factor of survival and long life in the context of the presence of a large number of age-related disorders in this group.

Keywords: inflammaging, cytokines, cell culture, aging phenotype, markers of aging

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Compliance with ethical standards: the study was approved by the Ethics Committee of the Pirogov Russian National Research Medical University (protocol No. 214 dated 13 December 2021). The subjects submitted the informed consent to participation in the study and the consent to data processing.

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ОПРЕДЕЛЕНИЕ ФЕНОТИПА СТАРЕНИЯ ПО ИЗМЕНЕНИЮ СПОНТАННОЙ И ИНДУЦИРОВАННОЙ ПРОДУКЦИИ ИНТЕРЛЕЙКИНА-6 И ИНТЕРЛЕЙКИНА-10 *IN VITRO*

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В процессе старения изменения в иммунной системе сопровождаются развитием системного стерильного воспаления «inflammaging». Выделяют успешный и патологический фенотипы старения. Степень развития «inflammaging» во многом зависит от соотношения про- и противовоспалительных медиаторов, особенно ИЛ-6 и ИЛ-10. Целью исследования было провести анализ продукции ИЛ-6 и ИЛ-10 в культурах мононуклеарных клеток (МНК) периферической крови пациентов как возможного подхода определения фенотипа старения. В работу включены данные пациентов пожилого возраста (n = 80), старческого возраста (n = 100) и долгожителей (n = 30), среди которых выделены подгруппы с успешным и патологическим фенотипами, а также группы сравнения (молодых лиц). Проводили оценку коэффициента стимуляции (КС) по соотношению уровней стимулированной и спонтанной выработки цитокинов. Для успешного фенотипа в пожилом, старческом возрасте и у долгожителей выявлено снижение КС ИЛ-6 до 5,3 [2,2–14,3] (p < 0,01), 5,3 [3,01–7,8] (p < 0,01), 6,5 [5,2–14,1] соответственно, по отношению к группе сравнения, где показатель составил 17,6 [13,7–31,1] (p < 0,05). При патологическом фенотипе показатели КС ИЛ-6 исследуемых возрастных групп достоверно не отличались от группы сравнения. Для успешного фенотипа в старческом возрасте выявлено повышение КС ИЛ-10 до 6,9 [3,8–13,8] по отношению к уровню группы патологического фенотипа — 3,3 [2,0–5,9] (p < 0,01) и группы сравнения — 2,0 [1,9–2,2] (p < 0,001). В группе долгожителей при патологическом фенотипе значительно повышался КС ИЛ-10 (11,2 [5,4–18,1] против 2,7 [2,3–6,5] p < 0,001) в группе успешного старения, что может свидетельствовать о выраженном компенсаторном противовоспалительном резерве, являющемся фактором выживания и долголетия при наличии большого количества возраст-ассоциированных заболеваний в данной группе.

Ключевые слова: воспалительное старение, цитокины, культура клеток, фенотип старения, маркеры старения

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An increase in life expectancy and a dramatic increase in the share of elderly people in the structure of the population attract a lot of interest to the studies of aging mechanisms. Aging affects all systems of the body, including the immune system that contributes greatly to shaping the aging trajectories. The inflammaging theory is currently one of the key theories of aging [1]. According to this theory, the chronic sterile low-grade inflammation is developed during aging, which can contribute to the age-associated disorders. Various factors, such as oxidative stress, impaired autophagy, emergence of senescent cells, microbiota composition alteration, inflammasome activation, etc., can cause such inflammation [2]. All the above factors lead to the increase in DAMP (damage-associated molecular patterns) and PAMP (pathogen-associated molecular patterns), and, therefore, to the increased production of pro-inflammatory cytokines, chemokines. Two aging phenotypes are distinguished based on the inflammation severity: successful and pathological aging. Successful aging is associated with reaching the optimal levels of physical, cognitive, and psycho-social adaptation in the elderly. Pathological aging, in contrast, is associated with faster aging, development of age-associated disorders resulting in disability and decline in quality of life [3]. In this regard, the search for approaches determining the aging phenotype is a relevant task.

There are extensive data on the cytokine system alteration during aging in the literature [4, 5]. However, there are currently almost no papers considering alterations of pro- and anti-inflammatory cytokines in broad age range in terms of successful and pathological aging phenotypes. It has been shown that the pathological aging phenotype in senile individuals is characterized by the increase in serum concentrations of pro-inflammatory cytokines (IL6, TNF, IL18), while the levels of anti-inflammatory cytokines, such as IL10 and TGF, in contrast, decrease compared to that of young adults [6]. However, systemic cytokine levels do not answer the question, how the immune system cells will respond to infectious stimuli or damage, which can be important in the context of assessing the prognosis of the infectious disease, such as COVID-19, or the course of the age-associated disorder. Furthermore, the levels of the most indicative cytokines, such as IL6 and IL10, in peripheral blood of patients showing no clinical manifestations of systemic inflammation, are still low, literally on the lower sensitivity limit of modern diagnostic test systems, which makes assessment of these indicators in clinics as the diagnostic and prognostic inflammaging markers rather controversial. In this regard, the analysis of the spontaneous and induced cytokine production by mononuclear cells (MNCs) in in vitro cultures seems to be more informative in terms of determining the aging phenotype.

The study aimed to perform the analysis of spontaneous and bacterial lipopolysaccharide (LPS)-induced IL6 and IL10 production in the cultures of peripheral blood MNCs from patients of older age groups with different aging phenotypes.

METHODS

The following subjects were included in the study:

– 80 elderly individuals (average age 68.7 ± 4.3 years, among them 53 females, 27 males);

– 100 senile patients (average age 81.8 ± 5.3 years, among them 72 females, 28 males);

– 30 centenarians (average age 92.7 ± 1.7 years, among them 21 females, 9 males).

All the patients were examined at the Geriatric Center, Moscow Diagnostic Clinical Center No. 1.

Exclusion criteria: acute disorder or exacerbation of chronic disorder at the time of enrollment. Inclusion criteria: subject's age corresponding to the studied group; availability of the submitted informed consent to participation in the study.

To assess the aging phenotype, the comprehensive geriatric assessment (CGA) was conducted that included evaluation of somatic status (Charlson Comorbidity Index, CCI), physical health (Short Physical Performance Battery, SSPB), and cognitive functions (Mini-Mental State Examination, MMSE). The criteria for dividing into subgroups with successful and pathological aging are provided in the Table.

The comparison group included 25 young adults (average age 22.4 ± 2.8 years), among them 16 females and 9 males. All the young adults enrolled had no acute or chronic disorder at the time of enrollment, as well as no limitation of physical functioning or cognitive impairment. Inclusion criteria: subjects in young adulthood; availability of the submitted informed consent to participation in the study. Exclusion criteria: acute or chronic disorder.

Peripheral blood samples were used as biomaterial for the study.

MNCs for culturing were isolated from peripheral blood on the Ficoll–Urografin density gradient medium (p = 1.077 g/cm3) [8]. The cells isolated were cultured for 24 h in the RPMI-1640 medium (Capricorn cop., USA) supplemented with the 20% fetal calf serum (Panexin, GMbH, Germany) at the temperature of 37 °C and 5% CO_2. The concentration of cells during culturing was 1×10^6 cells/mL. To assess the stimulated cytokine production, a bacterial LPS (Servicebio, China) with the nominal concentration of 0.1 μg/mL was added to the cells. Spontaneous cytokine production was assessed in the cell culture not supplemented with the LPS or other stimulator.

The IL6 and IL10 cytokine concentration was determined in the cell culture supernatant by enzyme-linked immunoassay using the commercially available test systems (Vector-Best, Russia).

Statistical data processing was performed using the Microsoft Excel (Microsoft Inc., USA) and GraphPad Prism 4.0 (GraphPad Software Inc., USA) software packages. The Kruskall–Wallis Test with the post-hoc Dunn's Multiple Comparison Test was used to assess the differences between the studied groups. The intergroup differences were considered significant with the p-value below 0.05 (significance level $\alpha = 0.05$).

RESULTS

Our findings show a significant increase in basal production of both IL6 and IL10 in all the studied groups relative to the comparison group of young adults, where the levels of these

Table. Criteria for dividing the studied groups into subgroups with successful and pathological aging

	Elderly age		Senile age		Centenarians	
Criteria	Successful aging	Pathological aging	Successful aging	Pathological aging	Successful aging	Pathological aging
CCI	2–3	4+	4–6	7+	4–8	9+
SSPB	11–12	1–10	9–12	0–8	8+	0–5
MMSE	28–30	below 28	28–30	below 28	24–30	below 24*

Note: * — based on the data [7].

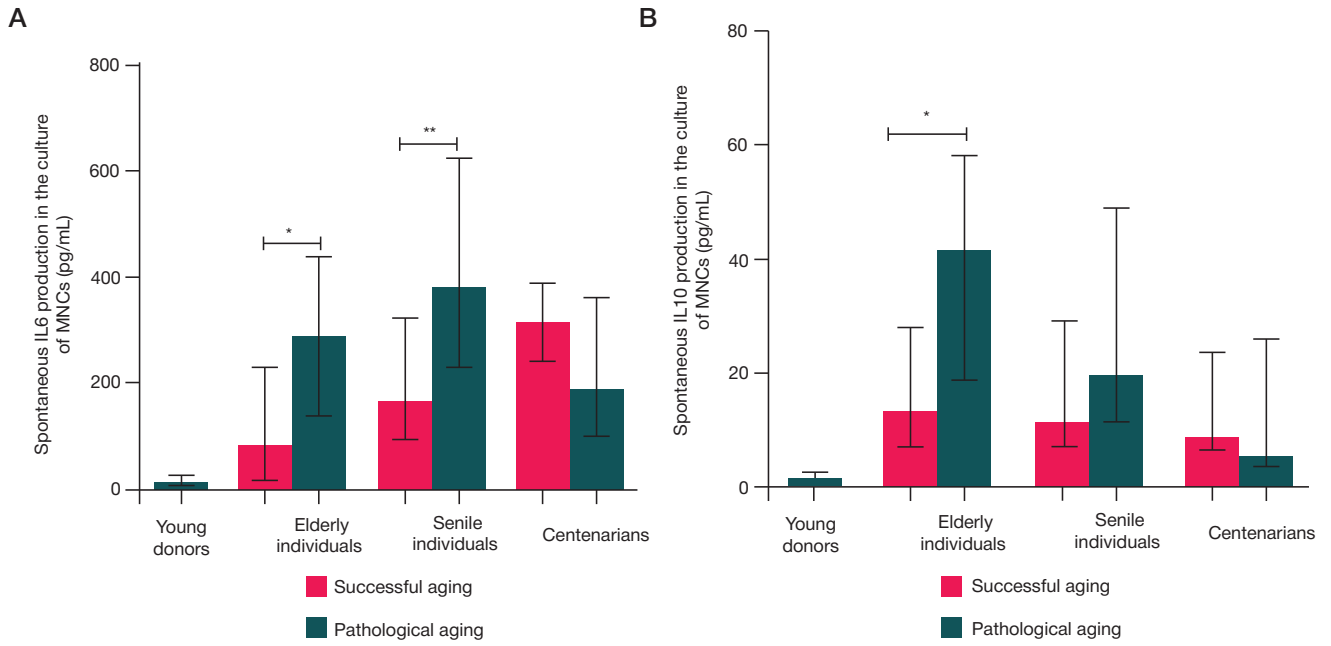

Fig. 1. Spontaneous IL6 **(A)** and IL10 **(B)** production in the culture of peripheral blood MNCs of elderly individuals, senile individuals, and centenarians with different aging phenotypes. The data are presented as the median (Me) and interquartile range, significant differences between aging phenotypes within the studied age groups: * — $p < 0.05$; ** — $p < 0.01$

cytokines were 12.5 [7.7–23.2] pg/mL and 1.4 [0.8–2.2] pg/mL, respectively (Fig. 1). Furthermore, the IL-6 levels that are elevated compared to the successful phenotype are also typical for elderly and senile individuals in the subgroups with pathological aging phenotype (Fig. 1A). Thus, in elderly individuals with the pathological aging phenotype, the levels of spontaneous IL6 production reached 289 [138–437] pg/mL, while that reported for the successful phenotype were 84.8 [19.4–232] pg/mL; in senile age, these were 377 [225–624] pg/mL for the pathological aging phenotype and 163 [97.3–319] pg/mL for the successful phenotype, respectively. No significant differences between phenotypes were reported for the group of centenarians. At the same time, the increase in the IL10 cytokine levels to 41.1 [18.5–58.1] pg/mL compared to the successful phenotype (13.2 [7.3–27.9] pg/mL) was typical for the pathological phenotype in the group of elderly individuals. Only the upward trend of IL10 levels was reported for the group of senile individuals (Fig. 1B).

In addition to assessment of spontaneous cytokine production by the peripheral blood MNCs, the cells were stimulated with the bacterial LPS. Such an approach can be considered as the method to assess potential pro- and anti-inflammatory activity of the innate immunity cells.

The stimulated IL6 production is increased in all groups, it shows the same general pattern of changes, as the spontaneous production (Fig. 2A).

However, the IL10 measurement results were more interesting (Fig. 2B). Thus, the subgroup of senile patients with successful aging showed the significantly ($p < 0.05$) higher levels (117.3 [61.3–318.2] pg/mL) compared to the

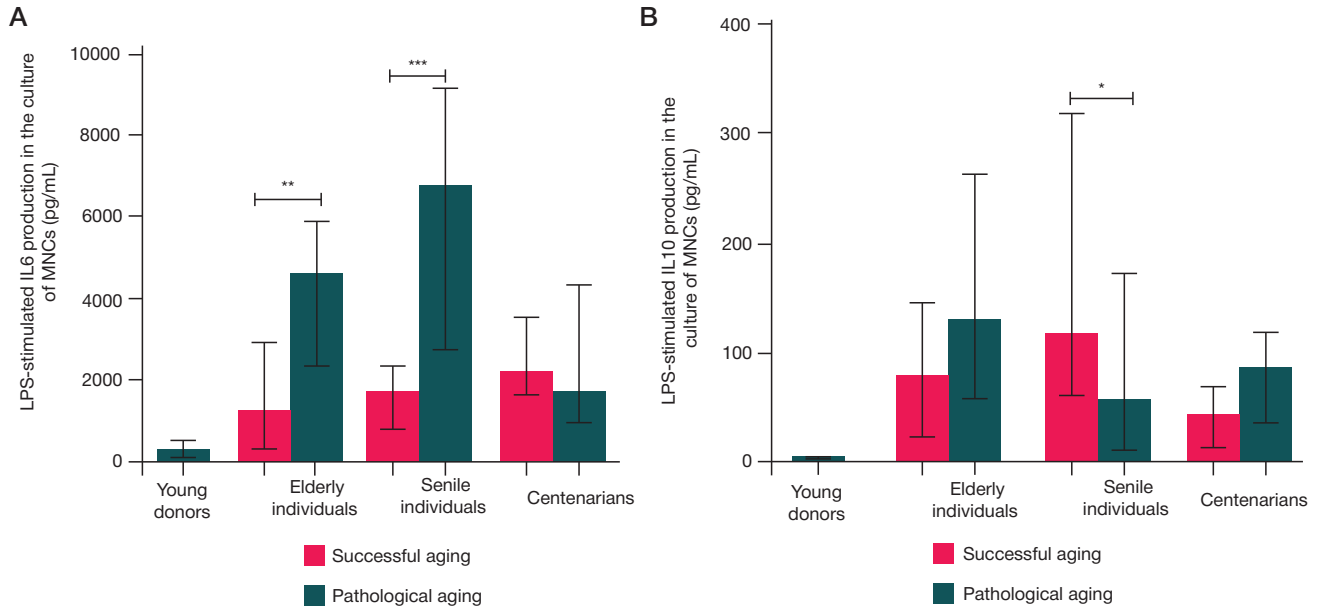

Fig. 2. LPS-induced IL6 **(A)** and IL10 **(B)** production in the culture of peripheral blood MNCs of elderly individuals, senile individuals, and centenarians with different aging phenotypes. The data are presented as the median (Me) and interquartile range, significant differences between aging phenotypes within the studied age groups: * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$

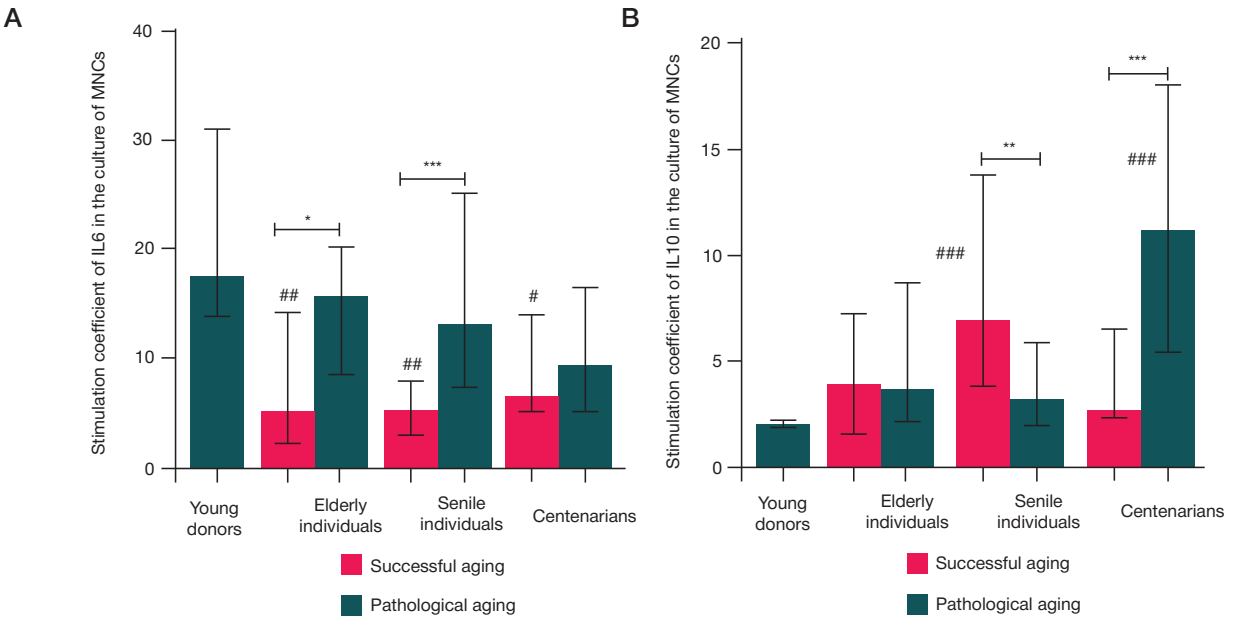

Fig. 3. Stimulation coefficients for IL6 **(A)** and IL10 **(B)** production in the culture of peripheral blood MNCs of elderly individuals, senile individuals, and centenarians with different aging phenotypes. The data are presented as the median (Me) and interquartile range, significant differences between aging phenotypes: * — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$; significant differences relative to the comparison group (young donors): # — $p < 0.05$; ## — $p < 0.01$; ### — $p < 0.001$

subgroup with the pathological phenotype, where the values were 57.4 [10.8–172.3] pg/mL. No significant differences in IL10 production were revealed in the elderly patients and centenarians with different aging phenotypes.

For better interpretation of these data as an integral indicator of the spontaneous and stimulated cytokine production, the stimulation coefficient (SC) was used, representing the ratio of the stimulated cytokine production to its spontaneous production by peripheral blood MNCs. This coefficient can be considered as an indicator that the cells are ready to produce pro- and anti-inflammatory cytokines in response to PAMP and DAMP [9]. The increase in the SC of this or that cytokine can indicate the increased readiness to cytokine production, while the decrease can indicate the decreased readiness.

The SC for IL6 (SCIL-6) reaches its maximum in the comparison group — 17.6 [13.7–31.1] (Fig. 3A), which suggests high cell reactivity in young adulthood. Furthermore, successful aging phenotype demonstrates a significant SCIL-6 decrease with age, which reflects the immunoaging adaptive nature. In elderly individuals, the SCIL-6 values were 5.3 [2.2–14.3], in senile individuals these were 5.3 [3.01–7.8], in centenarians these were 6.5 [5.2–14.1]. Despite the fact that SCIL-6 demonstrates a downward trend in the elderly and senile individuals with the pathological phenotype, it shows no significant differences from that of young adults and remains significantly increased relative to the indicator reported for the successful phenotype. Centenarians represent a special group, where the presence of the age-associated disorder did not prevent longevity; the most prominent SCIL-6 decrease was reported for centenarians with both successful and pathological aging phenotypes. The SC for IL10 (SCIL-10) demonstrates the opposite changes (Fig. 3B). With age, the increase in this indicator was observed compared to the group of young adults, where it was 2.0 [1.9–2.2]. However, in elderly individuals, no significant differences compared to young adults or between the aging phenotypes are observed. Given high readiness for IL6 production by the cells in individuals of this age with pathological aging, this can be interpreted as imbalance in pro- and anti-inflammatory signals, which represents the sign of the developing inflammaging [10]. In senile age, such imbalance is much more prominent: multidirectional SC changes in the groups with successful and pathological aging are reported for IL6 and IL10. In the subgroup with successful aging, SCIL-10 was increased to 6.9 [3.8–13.8] vs. 3.3 [2.0–5.9] revealed in individuals with the pathological aging phenotype. In the group with pathological aging, SCIL-6 turned out to be increased — 13.2 [7.4–25.3] vs. 5.3 [3.01–7.8] in the subgroup with successful aging. In the group of centenarians, SCIL-10 was significantly increased in the subgroup with pathological aging (11.2 [5.4–18.1]), while in individuals with successful aging the value was 2.7 [2.3–6.5], it did not differ from the value of the comparison group.

Study limitation

This study did not consider the impact of gender on the findings, as well as the impact of the presence of certain age-associated disorders in patients of the studied groups.

DISCUSSION

Numerous studies of systemic cytokine levels show that elevated concentrations of pro-inflammatory cytokines, such as IL1b, TNF, IL18, and IL6, in blood are associated with geriatric syndromes, such as senile asthenia, sarcopenia, etc. [11], as well as with the more severe course of the age-associated disorder and in some cases with the risk of death [12]. Furthermore, the available data on anti-inflammatory cytokines are less informative. As for IL10, it is well known that the increase in IL10 levels is not associated with the presence of disorders or geriatric syndromes [13].

According to the research, elevated systemic levels of anti-inflammatory cytokines IL10 [14] and TGF-β [15] (compared to senile individuals) are typical for centenarians; our previous findings are also in line with these data [2]. However, these data are hardly applicable for the elderly and senile age groups, since the levels of anti-inflammatory cytokines turn out to be low and show no relationship with the age-associated disorder.

A slow increase in pro-inflammatory cytokine levels that is asymptomatic and referred to as low-grade inflammation is

typical for inflammaging. At the same time, the development of infectious disease or exacerbation of chronic non-communicable disease results in the fact that the inflammatory response reaches the clinically significant level. However, the immune system reactivity is commonly not assessed in such situations. Assessment of the IL10 and IL6 production in vitro can be considered as assessment of such reactivity.

The increase in spontaneous IL10 and IL6 production in MNC cultures *in vitro* in individuals of older age groups relative to young donors can be interpreted as the fact of pre-stimulation of cells at the organism level resulting from the increase in the PAMP and DAMP circulating molecular patterns [16]. This is in line with the available literature data. In particular, it has been shown that both basal and stimulated TNF production by the peripheral blood cells increases with age [17]. Such stimulation is more prominent in elderly and senile individuals with the pathological aging phenotype. In the group of elderly individuals, a significant increase in IL10 levels also attracts attention, which can be considered as a compensatory response that inhibits pro-inflammatory activity. No significant differences in spontaneous cytokine production have been revealed in the group of centenarians with different phenotypes.

LPS stimulation of cells leads to the increased production of both IL6 and IL10 in all age groups, exceeding the cytokine levels of young individuals under the same conditions. However, the pathological aging phenotype is characterized by higher IL6 compared to the successful phenotype even in elderly individuals, which suggests the onset of inflammaging and setting an unfavorable trajectory of the aging process [18]. In senile individuals with the pathological phenotype, these manifestations are complemented by the decreased stimulated IL10 production relative to the successful aging phenotype.

The analysis of the SC as an integral indicator reflecting cell reactivity during cytokine production shows that with the successful aging phenotype all the older age groups, including centenarians, show the SCIL-6 decrease compared to young individuals. This can be considered as a favorable adaptive process that accompanies aging and shows that, despite the general increase in basal IL6 production, the readiness for further pro-inflammatory activity is limited, which inhibits inflammaging. While the lack of reactivity or insufficient decrease in reactivity in relation to IL6 production with age suggests unfavorable course of aging. Furthermore, SCIL-10, indicating readiness for IL10 production, in contrast, shows an upward trend with age. Despite the fact that no significant differences in SCIL-10 between aging phenotypes and compared to young adults have been reported for elderly people; in senile age, elevated SCIL-10 indicates the successful aging phenotype.

It has been noted that centenarians represent a special group, where we see a significant SCIL-10 increase specifically in the group with pathological aging, which can play a role of counterweight to the inflammaging processes in this group being one of possible factors associated with the longevity of these people [4]. Thus, we can say that a successful aging trajectory looks like the decreased readiness to IL6 production and increased readiness to IL10 production. Similar data were obtained for the long-living model rats, in which the increase in basal and stimulated IL10 expression by peritoneal macrophages was revealed, while similar IL6 levels and levels of other pro-inflammatory cytokines were lower, than in senile animals of control lineages [19].

Senile age is likely to be a critical period, when imbalance of pro- and anti-inflammatory factors, that has emerged in the elderly, results in severe manifestations of the pro-inflammatory aging phenotype [20, 21], such as rapid development of the age-associated disorder and aging manifestations affecting both physical performance and cognitive functions.

Despite the fact that the increased anti-inflammatory activity, specifically that related to IL10, can be a risk factor of both infectious diseases and cancer, it is of positive nature, since it is an inflammaging inhibitor. Thus, pro- and anti-inflammatory cytokines demonstrate their dual nature, serving as both protective and pathogenetic factors. Inflammation is a key component of immune responses to a pathogen. However, excess inflammation can result in numerous non-communicable diseases and increase the associated mortality rate. In terms of evolution, the immune system is optimized to fight infections in young adulthood, when pro-inflammatory responses are critical for survival. However, in the later age period excess inflammation becomes a risk factor of age-associated disorders, while inflammation limitation involving the anti-inflammatory cytokine system can contribute to longevity.

CONCLUSIONS

The aging process is accompanied by various changes in the body, including the immune system alterations. The immune system aging and inflammaging represent two inseparable processes inevitably launched in the body with age. Depending on external environmental factors and genetic predisposition, these shape the course of senility. If the body continues to adequately respond to external and internal danger signals (PAMP and DAMP) during aging and the associated changes in the innate immune system, then we can talk about the successful aging type, which in turn is accompanied by the anti-inflammatory status predominance, which compensates for excessively enhanced inflammatory activity. In the case where the threshold of adequate response is overcome, and the body responds with excessive production of pro-inflammatory mediators, without compensating for this with anti-inflammatory components, a pathological type of aging develops. We have developed an approach to the aging phenotype assessment based on determination of the stimulation coefficient representing the ratio of the stimulated cytokine production by peripheral blood MNCs to the spontaneous production. This coefficient can be considered as an indicator of inflammaging. The decrease in SC of IL6 with the increase in SC of IL10 was reported in the elderly and senile individuals with the successful aging phenotype. In the group of centenarians with the pathological aging phenotype, a significantly increased SC of IL10 was reported, which suggests a large compensatory anti-inflammatory reserve being the factor of survival and longevity in polymorbid individuals.

Functional assessment of the innate immunity cell pro-/anti-inflammatory activity can provide the basis for the personalized approach to prevention of age-associated disorders focused on maintaining adequate conditions of the external and internal environment, careful inflammation suppression, including pharmacological suppression, and shapes the possibility of active longevity.

References

1. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000; 908: 208–18. Available from: https://doi.org/10.1111/j.1749-6632.2000.tb06651.x.
2. Gankovskaya LV, Artemyeva OV, Namazova-Baranova LS, et al. Immunologicheskie aspekty starenija i vozrast-associirovannaja patologija. M.: Pediatr, 2021; 156 s. Russian.
3. Franceschi C, Capri M, Monti D, et al. Inflammaging and anti-nflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mechanisms of Ageing and Development. 2007; 128: 92–105. DOI: 10.1016/j.mad.2006.11.016.
4. Minciullo PL, Catalano A, Mandraffino G, Casciaro M, Crucitti A, Maltese G, et al. Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity. Arch Immunol Ther Exp. 2016; 64: 111–26. DOI: 10.1007/s00005-015-0377-3.
5. Puzianowska-Kuznicka M, Owczarz M, Wieczorowska-Tobis K, Nadrowski P, Chudek J, et al. Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. Immun Aging. 2016; 13: no.21. DOI: 10.1186/s12979-016-0076-x.
6. Grechenko VV, Artemyeva OV, Gromova TV, Gankovskaya LV. Izmenenie urovnej pro- i protivovospalitel'nyh citokinov u lic starcheskogo vozrasta i dolgozhitelej pri razlichnyh fenotipah starenija. Immunologija. 2024; 45 (5): 594–603. Available from: https://doi.org/10.33029/1816-2134-2024-45-5-594-603. Russian. Available from: https://doi.org/10.33029/1816-2134-2024-45-5-594-603.
7. Hayashida DY, Jacinto AF, Araújo LMQ, Almada Filho CMDI, Tommaso AB, Cendoroglo MS. Association between baseline Mini-Mental State Examination score and dementia incidence in a cohort of oldest old. Arq Neuropsiquiatr. 2021; 79 (12): 1090–4. DOI: 10.1590/0004-282X-ANP-2020-0543.
8. Zhu W, Revu S, Chen C, Dahl M, Ramkumar A, Kelly C, et al. Aging-dependent change in Th17 and cytokine response in multiple sclerosis. J Neuroinflammation. 2025; 22 (1): 150. DOI: 10.1186/s12974-025-03474-8. PMID: 40474243; PMCID: PMC12142853.
9. Bogatyreva AI, Gerasimova EV, Kirichenko TV, Markina YV, Popkova TV, Shalygina MV, et al. Proinflammatory Activation of Monocytes in Patients with Immunoinflammatory Rheumatic Diseases. Dokl Biochem Biophys. 2024; 517 (1): 228–34. DOI: 10.1134/S1607672924700959. Epub 2024 Jul 13. PMID: 39002011.
10. Franceschi C, Salvioli S, Garagnani P, de Eguileor M, Monti D, Capri M. Immunobiography and the heterogeneity of immune responses in the elderly: a focus on inflammaging and trained immunity. Front in Immunology. 2017; 8: 982. Available from: https://doi.org/10.3389/fimmu.2017.00982.
11. Pinti M, et al. A Comprehensive Analysis of Cytokine Network in Centenarians. Int J Mol Sci. 2023; 24 (3): 2719. DOI: 10.3390/ijms24032719.
12. Tylutka A, Walas L, Zembron-Lacny A. Level of IL-6, TNF, and IL-1b and age-related diseases: a systematic review and meta-analysis. Front Immunol. 2024; 15: 1330386. DOI: 10.3389/fimmu.2024.1330386.
13. Arosio B, Ferri E, Mari D, Tobaldini E, Vitale G, Montano N. The influence of inflammation and frailty in the aging continuum. Mech Ageing Dev. 2023; 215:111872. DOI: 10.1016/j.mad.2023.111872).
14. Franceschi C. Inflammaging as a major characteristic of old people: can it be prevented or cured. Nutr Rev. 2007; 65 (12 Pt 2): 173–76. DOI: 10.1111/j.1753-4887.2007.tb00358.x.
15. Zhou L, Ge M, Zhang Y, Wu X, Leng M, Gan C, et al. Centenarians Alleviate Inflammaging by Changing the Ratio and Secretory Phenotypes of T Helper 17 and Regulatory T Cells. Front Pharmacol. 2022; 13: 877709. DOI: 10.3389/fphar.2022.877709.
16. Fulop T, Larbi A, Pawelec G, Khalil A, Cohen AA, Hirokawa K, et al.. Immunology of Aging: the Birth of Inflammaging. Clinical Reviews in Allergy & Immunology. 2023; 64: 109–22. DOI: https://doi.org/10.1007/s12016-021-08899-6.
17. Bailey KL, Smith LM, Heires AJ, Katafiasz DM, Romberger DJ, LeVan TD. Aging leads to dysfunctional innate immune responses to TLR2 and TLR4 agonists. Aging Clin Exp Res. 2019; 31 (9): 1185–93. DOI: 10.1007/s40520-018-1064-0).
18. Olivieri F, Prattichizzo F, Lattanzio F, Bonfigli AR, Spazzafumo L. Antifragility and antiinflammaging: Can they play a role for a healthy longevity? Ageing Research Reviews. 2023; 84: 101836. DOI: 101836https://doi.org/10.1016/j.arr.2022.101836.
19. Dimitrijević M, Aleksić I, Vujić V, Stanojević S, Pilipović I, von Hörsten S, et al. Peritoneal exudate cells from long-lived rats exhibit increased IL-10/IL-1β expression ratio and preserved NO/urea ratio following LPS-stimulation in vitro. Age (Dordr). 2014; 36 (4): 9696. DOI: 10.1007/s11357-014-9696-2).
20. Silva R, Travassos L, Dutra F. The dichotomic role of single cytokines: Fine-tuning immune responses. Cytokine. 2024; 156408. DOI: https://doi.org/10.1016/j.cyto.2023.156408.
21. Silva R. The dichotomic role of cytokines in aging. Biogerontology. 2025; 26: 17. Available from: https://doi.org/10.1007/s10522-024-10152-4.

Литература

1. Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci. 2000; 908: 208–18. Available from: https://doi.org/10.1111/j.1749-6632.2000.tb06651.x.
2. Ганковская Л. В., Артемьева О. В., Намазова-Баранова Л. С. и др. Иммунологические аспекты старения и возраст-ассоциированная патология. М.: ПедиатрЪ, 2021; 156 с.
3. Franceschi C, Capri M, Monti D, et al. Inflammaging and anti-nflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mechanisms of Ageing and Development. 2007; 128: 92–105. DOI: 10.1016/j.mad.2006.11.016.
4. Minciullo PL, Catalano A, Mandraffino G, Casciaro M, Crucitti A, Maltese G, et al. Inflammaging and Anti-Inflammaging: The Role of Cytokines in Extreme Longevity. Arch Immunol Ther Exp. 2016; 64: 111–26. DOI: 10.1007/s00005-015-0377-3.
5. Puzianowska-Kuznicka M, Owczarz M, Wieczorowska-Tobis K, Nadrowski P, Chudek J, et al. Interleukin-6 and C-reactive protein, successful aging, and mortality: the PolSenior study. Immun Aging. 2016; 13: no.21. DOI: 10.1186/s12979-016-0076-x.
6. Греченко В. В., Артемьева О. В., Громова Т. В., Ганковская Л. В. Изменение уровней про- и противовоспалительных цитокинов у лиц старческого возраста и долгожителей при различных фенотипах старения. Иммунология. 2024; 45 (5): 594–603. Available from: https://doi.org/10.33029/1816-2134-2024-45-5-594-603.
7. Hayashida DY, Jacinto AF, Araújo LMQ, Almada Filho CMDI, Tommaso AB, Cendoroglo MS. Association between baseline Mini-Mental State Examination score and dementia incidence in a cohort of oldest old. Arq Neuropsiquiatr. 2021; 79 (12): 1090–4. DOI: 10.1590/0004-282X-ANP-2020-0543.
8. Zhu W, Revu S, Chen C, Dahl M, Ramkumar A, Kelly C, et al. Aging-dependent change in Th17 and cytokine response in multiple sclerosis. J Neuroinflammation. 2025; 22 (1): 150. DOI: 10.1186/s12974-025-03474-8. PMID: 40474243; PMCID: PMC12142853.
9. Bogatyreva AI, Gerasimova EV, Kirichenko TV, Markina YV, Popkova TV, Shalygina MV, et al. Proinflammatory Activation of Monocytes in Patients with Immunoinflammatory Rheumatic Diseases. Dokl Biochem Biophys. 2024; 517 (1): 228–34. DOI: 10.1134/S1607672924700959. Epub 2024 Jul 13. PMID: 39002011.
10. Franceschi C, Salvioli S, Garagnani P, de Eguileor M, Monti D, Capri M. Immunobiography and the heterogeneity of immune responses in the elderly: a focus on inflammaging and trained immunity. Front in Immunology. 2017; 8: 982. Available from: https://doi.org/10.3389/fimmu.2017.00982.
11. Pinti M, et al. A Comprehensive Analysis of Cytokine Network in Centenarians. Int J Mol Sci. 2023; 24 (3): 2719. DOI: 10.3390/ijms24032719.
12. Tylutka A, Walas L, Zembron-Lacny A. Level of IL-6, TNF, and IL-1b and age-related diseases: a systematic review and meta-analysis. Front Immunol. 2024; 15: 1330386. DOI: 10.3389/fimmu.2024.1330386.

13. Arosio B, Ferri E, Mari D, Tobaldini E, Vitale G, Montano N. The influence of inflammation and frailty in the aging continuum. Mech Ageing Dev. 2023; 215:111872. DOI: 10.1016/j.mad.2023.111872).
14. Franceschi C. Inflammaging as a major characteristic of old people: can it be prevented or cured. Nutr Rev. 2007; 65 (12 Pt 2): 173–76. DOI: 10.1111/j.1753-4887.2007.tb00358.x.
15. Zhou L, Ge M, Zhang Y, Wu X, Leng M, Gan C, et al. Centenarians Alleviate Inflammaging by Changing the Ratio and Secretory Phenotypes of T Helper 17 and Regulatory T Cells. Front Pharmacol. 2022; 13: 877709. DOI: 10.3389/fphar.2022.877709.
16. Fulop T, Larbi A, Pawelec G, Khalil A, Cohen AA, Hirokawa K, et al. Immunology of Aging: the Birth of Inflammaging. Clinical Reviews in Allergy & Immunology. 2023; 64: 109–22. DOI: https://doi.org/10.1007/s12016-021-08899-6.
17. Bailey KL, Smith LM, Heires AJ, Katafiasz DM, Romberger DJ, LeVan TD. Aging leads to dysfunctional innate immune responses to TLR2 and TLR4 agonists. Aging Clin Exp Res. 2019; 31 (9): 1185–93. DOI: 10.1007/s40520-018-1064-0).
18. Olivieri F, Prattichizzo F, Lattanzio F, Bonfigli AR, Spazzafumo L. Antifragility and antiinflammaging: Can they play a role for a healthy longevity? Ageing Research Reviews. 2023; 84: 101836. DOI: 101836https://doi.org/10.1016/j.arr.2022.101836.
19. Dimitrijević M, Aleksić I, Vujić V, Stanojević S, Pilipović I, von Hörsten S, et al. Peritoneal exudate cells from long-lived rats exhibit increased IL-10/IL-1β expression ratio and preserved NO/urea ratio following LPS-stimulation in vitro. Age (Dordr). 2014; 36 (4): 9696. DOI: 10.1007/s11357-014-9696-2).
20. Silva R, Travassos L, Dutra F. The dichotomic role of single cytokines: Fine-tuning immune responses. Cytokine. 2024; 156408. DOI: https://doi.org/10.1016/j.cyto.2023.156408.
21. Silva R. The dichotomic role of cytokines in aging. Biogerontology. 2025; 26: 17. Available from: https://doi.org/10.1007/s10522-024-10152-4.

COMPARATIVE PHARMACOKINETICS AND BIODISTRIBUTION OF HAEE AND HASS PEPTIDES

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Due to the limited availability of advanced methods to diagnose Alzheimer's disease, small molecules and short peptides capable of specifically biniding β-amyloid are of special interest. The study aimed to perform comparative assessment of pharmacokinetics and biodistribution of two model peptides, HAEE (His-Ala-Glu-Glu) and HASS (His-Ala-Ser-Ser), as potential platforms for the development of diagnostic kits, as well as to determine the relationship between the peptide structure and organotropism. The study involving BALB/c mice (*n* = 10) was conducted using the fluorescence labeled compounds. We used *in vivo* IVIS imaging, *ex vivo* fluorescence microscopy, and pharmacokinetic analysis. The results showed fundamental differences: the negatively charged HAEE was accumulated mainly in the kidney ($T_{½}β$ = 4.39 h) due to tubular reabsorption, while neutral HASS was soon captured by the liver ($T_{½}β$ = 2.76 h). The data obtained demonstrate the key role of amino acid composition in determining organotropism and open prospects for the development of organ-specific peptide systems.

Keywords: HAEE peptide, HASS peptide, BALB/c healthy mice, HAEE pharmacokinetics, HASS pharmacokinetics, *in vivo* fluorescence imaging (IVIS), blood brain barrier, HAEE biodistribution, HASS biodistribution

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Compliance with ethical standards: the study approved by the Ethics Committee of the Pirogov Russian National Research Medical University (protocol 03/2025 dated 23 January 2025) was conducted in accordance with the principles of Good Laboratory Practice (Order of the Ministry of Health of the Russian Federation No. 708n dated 23.08.2010, Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes).

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СРАВНИТЕЛЬНАЯ ФАРМАКОКИНЕТИКА И БИОРАСПРЕДЕЛЕНИЕ ПЕПТИДОВ HAEE И HASS

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В связи с ограниченной доступностью современных методов диагностики болезни Альцгеймера особый интерес представляют малые молекулы и короткие пептиды, способные специфично связываться с β-амилоидом. Целью работы было провести сравнительное изучение фармакокинетики и биораспределения двух модельных пептидов — HAEE (His-Ala-Glu-Glu) и HASS (His-Ala-Ser-Ser) как потенциальных платформ для разработки диагностических систем и установить взаимосвязь между структурой пептидов и их органотропностью. Исследование выполнено на мышах линии BALB/c (*n* = 10) с использованием флуоресцентно меченых соединений. Применяли IVIS-визуализацию *in vivo*, флуоресцентную микроскопию *ex vivo* и фармакокинетический анализ. Результаты выявили принципиальные различия: HAEE с отрицательным зарядом преимущественно накапливался в почках ($T_{½}β$ = 4,39 ч) благодаря реабсорбции в канальцах, тогда как нейтральный HASS быстро захватывался печенью ($T_{½}β$ = 2,76 ч). Полученные данные демонстрируют ключевую роль аминокислотного состава в определении органотропности и открывают перспективы для разработки органоспецифичных пептидных систем.

Ключевые слова: пептид HAEE, пептид HASS, BALB/c здоровые мыши, фармакокинетика HAEE, фармакокинетика HASS, *in vivo* флуоресцентная визуализация (IVIS), гематоэнцефалический барьер, биораспределение HAEE, биораспределение HASS

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Вклад авторов: А. В. Иванова — обзор литературы, экспериментальные исследования на мышиной модели BALB/c, проведение транскардиальной перфузии всех органов, подготовка рукописи; П. А. Лазарева — экспериментальные исследования на модели мышей BALB/c, проведение транскардиальной перфузии всех органов, анализ полученных результатов, подготовка рукописи; И. А. Кузьмичев — синтез флуоресцентного пептида HAEEGGGGK-Cy5 и HASSGGGGGK-Cy5; В. В. Вадехина — внутривенное введение пептида HAEEGGGGK-Cy5 и HASSGGGGGK-Cy5 через хвостовую вену, проведение транскардиальной перфузии всех органов; А. В. Косых — фиксация, подготовка гистологических срезов для съемки на микроскопе, съемка и подготовка рукописи; Н. Г. Гурская — получение изображений на флуоресцентном микроскопе и анализ, подготовка рукописи; М. А. Абакумов — постановка цели, разработка дизайна исследования, подготовка рукописи; все авторы внесли равнозначный вклад в подготовку публикации, подтверждают соответствие своего авторства международным критериям ICMJE.

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Alzheimer's disease remains one of the most socially significant neurodegenerative disorders and the main cause of dementia in elderly people [1, 2]. The disease prevalence grows exponentially with age: 53 new cases per 1000 people aged 65–74; 170 new cases per 1000 people aged 75–84, and 230 new cases per 1000 people aged over 85 [3, 4]. The disease pathogenesis is complex and multifaceted. However, this process is rather often accompanied by accumulation of β-amyloid (Aβ) in the form of senile plaques in the cerebral cortex [5]. Aβ represents a peptide with the length of 39–43 amino acid bases that is generated from the Aβ precursor protein (APP) [6]. This unique APP breakdown process provides important targets for treatment of Alzheimer's disease [7]. Two major peptides have the length of 40 ($Aβ_{40}$) and 42 ($Aβ_{42}$) amino acid bases. $Aβ_{42}$ is prone to aggregation *in vivo*, it is often considered to be more toxic [8, 9]. $Aβ_{42}$ accumulation triggers the cascade of abnormalities including formation of oligomers and fibrils, neuroinflammation activation, synaptic transmission impairment, neuronal death [6, 10, 11]. Unfortunately, the in-depth study of the amyloid hypothesis and other pathogenetic hypotheses has failed to produce any truly effective therapeutic strategy, while such strategies have been developed since the disease was first described in 1906 [12]. Modern methods to diagnose Alzheimer's disease are still inaccessible for widespread use due to technical complexity and significant expenses. In this regard, small molecules capable of specific binding to Aβ are of special interest; their versatility and the possibility of chemical modification open up prospects for the development of more affordable diagnostic platforms. Among such molecules, short peptide sequences, particularly tetrapeptides HAEE (His-Ala-Glu-Glu) and HASS (His-Ala-Ser-Ser) that combine high affinity for β-amyloid, inhibition of its aggregation, good pharmacokinetic properties, and the ability to cross the blood-brain barrier, turned out to be the most promising [13]. To ensure realization of their diagnostic potential, it is necessary to thoroughly assess pharmacokinetic properties, including biodistribution, the ability to cross the blood-brain barrier, and the dynamics of elimination from the body. This study is focused on comprehensive characterization of these parameters involving the use of the fluorescence labeled HAEE and HASS analogues in animal models.

The study aimed to determine pharmacokinetic parameters of the HAEE and HASS tetrapeptides in healthy animals, including half-life ($T_{1/2}$), in order to assess the tetrapeptide biodistribution, stability *in vivo*, and prospects for further use as a basis for radiopharmaceuticals or therapeutic agents.

METHODS

All the experiments were conducted at the research laboratory of the Deparment of Medical Nanobiotechnology, as well as at the Department of Regenerative Medicine of the Research Institute of Translational Medicine, Pirogov Russian National Research Medical University.

IVIS imaging *in vivo*/*ex vivo*

Female BALB/c mice aged 3 months with the weight of 20–25 g were purchased brom the breeding nursery of the Center of Biomedical Technology of the Federal Medical Biological Agency of Russia, Stolbovaya branch (Moscow region, Russia).

Mice had ad libitum access to food and water. The animals received extruded complete feed for laboratory animals: mice, rats, hamsters (Laborantsnab, Russia). Daytime was 12 h (between 7:00 and 19:00); illuminance during the light part of the cycle was 70–90 lx; the temperature in the room where the animals were permanently kept was 22 °C.

Each experiment involved five animals. The test peptides were HAEEGGGGK-Cy5 and HASSGGGGK-Cy5 (purity > 95% based on the HPLC data). The peptides were dissolved in the sterile 0.9 % NaCl solution to the final concentration of 250 μM before administration. The resulting solutions were filtered through the 0.22 μm filter and injected intravenously in the tail vein (n = 4 animals per group) in the amount of 100 μL. The control group was administered 100 μL of the 0.9% NaCl solution.

Intravital fluorescence imaging was performed using the IVIS Spectrum CT imaging system (Perkin Elmer, USA) at the time points of 15 min, 1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 24 h after administration. Fluorescence imaging was accomplished under inhalation anesthesia with 2% isoflurane (Aerrane, Baxter HealthCareCorporation, USA) mixed with air.

The animals were ansthesized by intraperitoneal injection of tiletamine (Zoletil, Virbac, France) for further experiments. Then transcardial perfusion was performed. For that the right atrium was incised, the cannula was inserted in the left ventricle, and blood channels were washed sequentially with the sterile phosphate buffered saline (Sigma-Aldrich, USA) and HistoSafe 10% buffered formalin (Biovitrum, Russia). The organs were retrieved, and *ex vivo* fluorescence imaging was performed.

Fluorescence analysis and kinetic modeling

Photon emission was calculated using the Living Image 4.3 software with selection of the region of interest (ROI). Fluorescence values of the control animal for each time point were subtracted from the animals' fluorescence values in order to eliminate systematic measurement errors. The same was done for the organs.

Widefield fluorescence microscopy

The 10 μm cryosections were cut using the HM525 cryo microtome (Thermo Scientific, USA). For further analysis,

Table 1. Dynamic changes in the mean fluorescence intensity (p/sec/cm^2/sr) injection of the HAEE and HASS fluorescence peptides

Time, h	HAEE (Mean ± SD)*	HASS (Mean ± SD)*
0.25	$(1.23 \pm 0.18) \times 10^9$	$(1.31 \pm 0.19) \times 10^9$
1	$(4.61 \pm 0.51) \times 10^8$	$(2.11 \pm 0.18) \times 10^8$
2	$(1.76 \pm 0.16) \times 10^8$	$(8.69 \pm 0.75) \times 10^7$
3	$(1.21 \pm 0.09) \times 10^8$	$(6.04 \pm 1.01) \times 10^7$
4	$(9.53 \pm 1.07) \times 10^7$	$(5.57 \pm 0.92) \times 10^7$
5	$(7.53 \pm 0.82) \times 10^7$	$(4.93 \pm 0.94) \times 10^7$
6	$(7.87 \pm 0.99) \times 10^7$	$(4.20 \pm 0.71) \times 10^7$
24	$(3.14 \pm 0.28) \times 10^7$	$(1.97 \pm 0.23) \times 10^7$

Note: * — the data are presented as the mean ± standard deviation (Mean ± SD) for n = 4

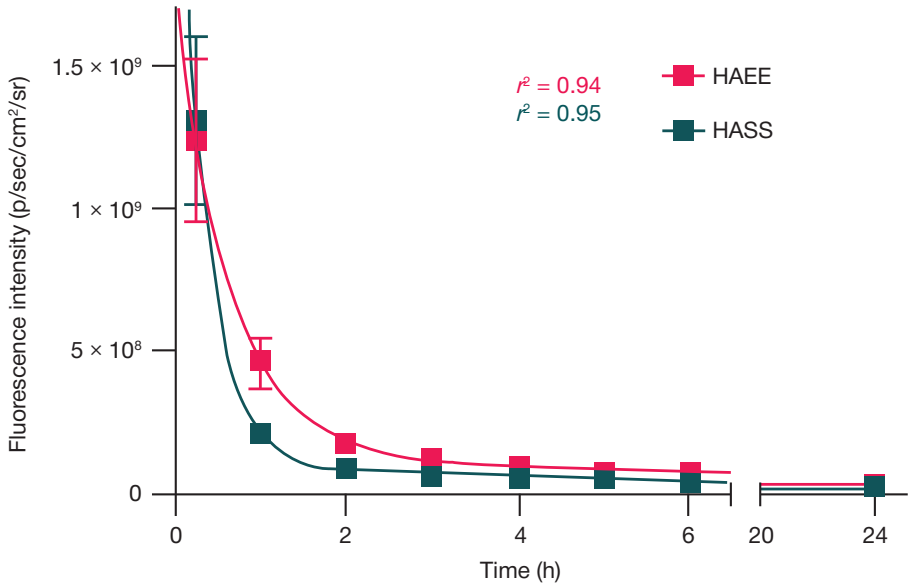


Fig. 1. *In vivo* fluorescence kinetic curves of the studied HAEE (r^2 = 0.94) and HASS (r^2 = 0.95) peptides

sections were fixed in the 10% buffered formalin (Biovitrum, Russia) for 15 min, sequentially triple washed with phosphate buffered saline containing no Ca^{2+} and Mg^{2+} (PanEco, Russia), and embedded in the VECTASHIELD Atifade Mounting Medium with DAPI (VectorLabs, USA). Images were acquired using the EVOS FL Auto imaging system (Thermo Scientific, USA).

Statistical analysis

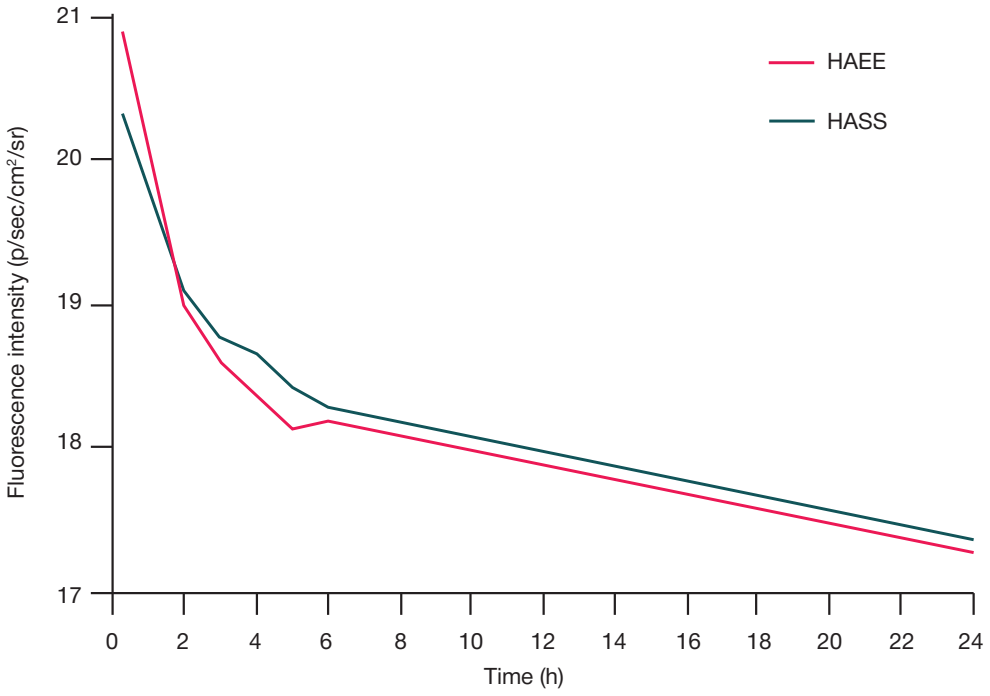
Data analysis was performed in GraphPad Prism 8.0.1 (GraphPad Software, USA). The graph of the fluorescence signal intensity as a function of time was presented as mean values with standard deviations and the trend line for the two-chamber model. The graph of ex vivo drug accumulation in the organs contained the mean value, maximum and minimum values for the group.

RESULTS

Teporary changes in fluorescence intensity were reported after a single intravenous injection of the HAEE and HASS fluorescence peptides to BALB/c mice (Table 1). Both compounds showed rapid systemic absorption reaching the maximum concentration 0.25 h after administration. Furthemore, HASS ((1.31 ± 0.19) × 10^9 p/sec/cm²/sr) showed the 6.5% higher fluorescence intensity compared to HAEE ((1.23 ± 0.18) × 10^9 p/sec/cm²/sr) with subsequent exponential decrease in indicators. The findings are more clearly demonstrated in Fig. 1.

Plotting of the resulting kinetic profiles on a logarithmic scale suggests the two-chamber model of the HASS and HAEE peptide elimination (Fig. 2).

The HAEE and HASS kinetic curves (Fig. 1, 2) described by the two-chamber model ($Ap = A_1 \cdot e^{-\alpha t} + A_2 \cdot e^{-\beta t}$) demonstrate



Fip. 2. Comparative kinetics of the HAEE and HASS peptide elimination

Table 2. Comparison of the HAEE and HASS pharmacokinetic parameters after intravenous administration

Parameter	HAEE	HASS
Rapid elimination phase, %	93.37	96.43
α, h	1.6	3.11
β, h	0.16	0.25
$T_{1/2}$ of the rapid distribution phase, h	0.43	0.22
$E_{1/2}$ of the elimination phase, h	4.39	2.76
A_1 of the rapid distribution phase, p/sec/cm²/sr	1.64×10^9	2.61×10^9
A_2 of the elimination phase, p/sec/cm²/sr	1.16×10^8	9.67×10^7
AUC (0→∞), h * p/sec/cm²/sr	3.69×10^9	1.51×10^9

considerable differences in elimination parameters (Table 2): HASS is characterized by the dominance of the rapid phase (96.43% vs. 93.37%), higher rate constant α (3.11 h^{-1} vs. 1.6 h^{-1}) and shorter $T_{½}α$ (0.22 h vs. 0.43 h), which is correlated to the steep initial decline on the graphs, while HAEE showing prolonged elimination with the extended $T_{½}β$ (4.39 h vs. 2.76 h) is characterized by the gentle slope on the kinetic curve and the large AUC (3.69×10^9 vs. 1.51×10^9 h· p/sec/cm²/sr), which suggests its potential advantage for the long diagnosis.

Ex vivo assessment of the HAEE and HASS peptide accumulation was performed 24 h after injection. According to the data obtained (Fig. 3), the peptides show different organ specificity: HAEE is localized mainly in the kidney, while HASS is localized mainly in the liver. Both compounds are accumulated in the lung and brain showing no significant intergroup differences ($p > 0.05$). As for the heart and spleen, fluorescence intensity in the HAEE group was significantly higher, than in the HASS group ($p < 0.05$).

The HAEE longer half-life results from the fact that it is eliminated mainly by the kidney, which has been confirmed by fluorescence microscopy of the sections that has revealed intense fluorescence signal accumulation in the renal tubular epithelium (Fig. 4), which is consistent with the IVIS data (Fig. 5), where the kidneys show stronger fluorescence signal compared to other organs within 24 h, while HASS is accumulated primarily in the liver (Fig. 5).

Assessment of representative microphotos of the mouse kidney tissue sections confirms increased HAEE content in the form of accumulation of the large number of Cy^{5+} aggregates in the cells of renal proximal tubules (Fig. 4A, B) compared to HASS (Fig. 4C, D).

DISCUSSION

Our studies have revealed considerable differences in biodistribution of the HAEE and HASS peptides, which can be explained by their structural and functional features. HAEE having low molecular weight (< 2 kDa) and weak negative charge demonstrates primarily renal excretion. Such process is typical for low molecular weight peptides [14], which are reabsorbed through pinocytosis after glomerular filtration. The presence of the Cyanine 5 (Cy5) fluorescent label in the peptide structure can increase its lipophilicity, contributing to penetration through the cell membranes, specifically in the epithelium of the renal proximal tubules, which explains the reported compound accumulation in the kidney tissue. Nor can the possibility be excluded that the peptide specifically interacts with the cellular receptors. However, this aspect requires further research. HAEE can enter the liver by passive transport through the membranes of hepatocytes and Kupffer cells. Furthermore, considering the key role of liver in metabolism and detoxification, accumulation of the peptide in this organ can be associated with its biotransformation or active transport via hepatocytes. Upon systemic administration, pulmonary epithelium is also permeable for small lipophilic molecules [15], which is confirmed by detection of fluorescence signal in the lung. Experimental studies involving BALB/c mice have shown

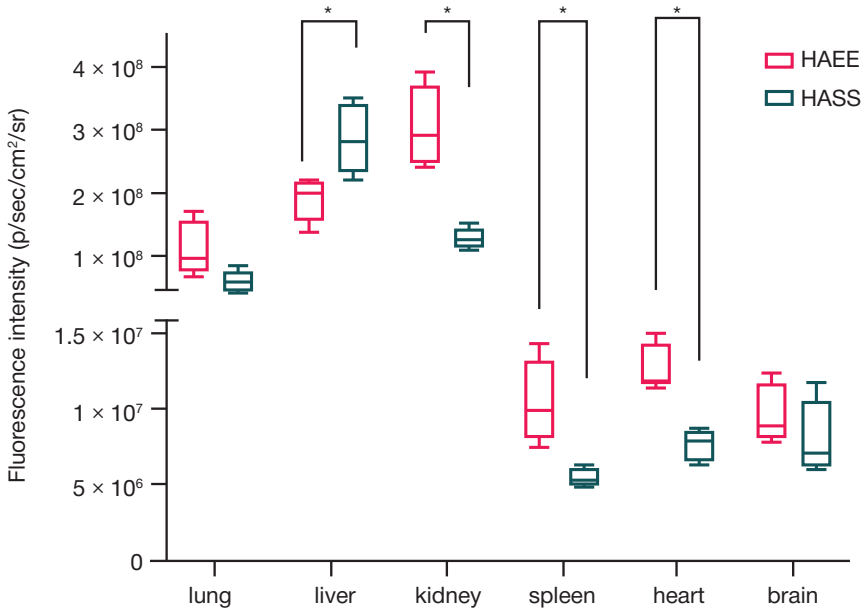


Fig. 3. *Ex vivo* assessment of the HAEE and HASS accumulation in the organs 24 h after intravenous administration. * — $p < 0.05$, significance of intergroup differences based on the Mann–Whitney U-test

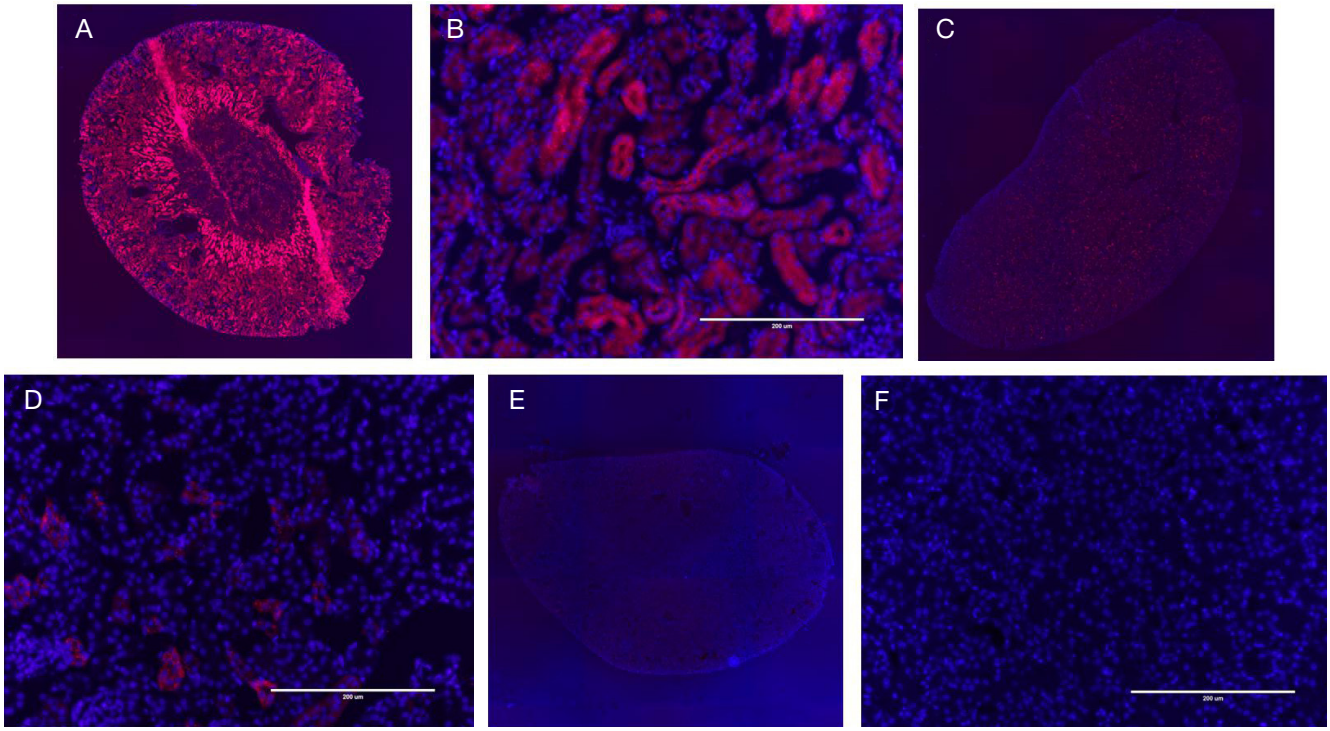


Fig. 4. Histological analysis of the mouse kidney tissue after the HAEE and HASS systemic administration. **A**, **B**. Localization of the Cy5-labeled HAEE (*red*) in the mouse kidney 24 h after intravenous administration. **A**. Giant section of the mouse kidney. **B**. Accumulation of fluorescent granules in epithelial cells of the renal proximal tubules. **C**, **D**. Localization of the Cy5-labeled HASS (*red*) in the mouse kidney 24 h after intravenous administration. **C**. Giant section of the mouse kidney. **D**. Accumulation of fluorescent granules in epithelial cells of the renal proximal tubules. **E**, **F**. Kidney of the control mice 24 h after intravenous administration of saline. **E**. Giant section of the kidney. **F**. Magnified image of the kidney tissue obtained by superimposing red and blue fluorescence. The nuclei are DAPI stained (*blue*). Scale bar 200 μm

that HAEE is characterized primarily by renal excretion, which is confirmed by three key observations: 1) high fluorescence intensity in the kidney 24 h after administration recorded by both IVIS imaging and fluorescence microscopy; 2) prolonged half-life ($T_{½}\beta$ = 4.39 h), which is explained by reabsorption in the renal tubules; 3) rapid initial elimination ($T_{½}\alpha$ = 0.43 h) suggesting that there are no stable complexes with blood plasma proteins.

In contrast to HAEE, the neutrally charged HASS peptide demonstrates fundamentally different pharmacokinetic characteristics. Its peak fluorescence signal is 6.5% higher than that of HAEE, which is likely to be due to reduced binding to the tissue structures. HASS is characterized by enhanced elimination with the half-life of $T_{½}\alpha$ = 0.22 h and $T_{½}\beta$ = 2.76 h resulting probably from active capture by hepatocytes. The ex vivo testing 24 h after administration confirmed selective accumulation of the peptide in the liver tissue.

Despite similar size (< 2 kDa), HAEE shows the 1.7 times better blood-brain barrier permeability (AUC (0→∞)), which is likely to be due to electrostatic effects and the features of interaction with transport systems.

The findings demonstrate considerable differences in the distribution of the studied peptides across organs. In particular, it has been found that HAEE is excreted primarily by the kidney, which determines its potential diagnostic value for renal disorders. In contrast to HAEE, HASS shows high hepatospecificity and high hepatic metabolism rate, which allows one to consider it as a promising basis for the development of the delivery systems targeting the liver. The presence of the Cy5 label increases lipophilicity of both peptides, which contributes to their transport through the cell membranes. However, the limited crossing of the blood-brain barrier (especially that of HASS) suggests the need for structural optimization for the use in neurodiagnosis. Further research is required for better understanding of the mechanisms underlying biodistribution of tetrapeptides: 1) identification of the transport systems (megalin/OATP (organic anion transporting polypeptide)); 2) complete metabolic profile in biological fluids; 3) correlation between structural alterations of peptides and their distribution across the organs.

CONCLUSIONS

Thus, the comparative study conducted has revealed fundamental differences in the HAEE and HASS behavior

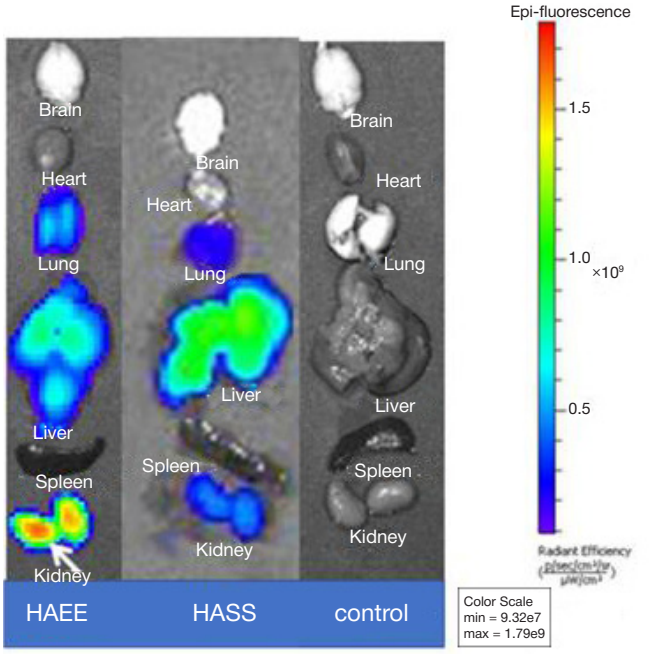


Fig. 5. HAEE and HASS organ specificity within 24 h (IVIS). *White arrow* points to the kidneys with maximum buildup. Color scale: from blue (min) to red (max)

in vivo. The peptides demonstrate no only different organ tropism, but also fundamentally different pharmacokinetic profiles (including half-life, $T_{1/2}$), which opens up the prospects for differentiated use of those in the diagnosis and targeted drug delivery. It has been found that HAEE with short $T_{1/2}$ and renal excretion is promising for the development of diagnostic radiopharmaceuticals, while HASS showing hepatotropism and rapid hepatic metabolism is of intrest in terms of developing the targeted delivery systems. It should be noted that the results were obtained using the healthy mouse model. These data that should be further tested in model transgenic animals with Alzheimer's disease now suggest new strategies for complex therapy combining the organ-specific peptide transport and pathogenetic effects. Furthermore, the impact of fluorescent label on pharmacokinetic parameters should be assessed separately.

References

1. Huang LK, Kuo HY, Chen HJ, et al. Clinical trials of new drugs for Alzheimer disease: a 2020–2023 update. J Dent Res. 2023; 88 (3): 1–19.
2. Facts D. Alzheimer's disease facts and figures report: executive summary. Alzheimers Dement. 2024; 20 (5): 40–42.
3. Adlard PA, Bush AI. A review of β-amyloid neuroimaging in Alzheimer's disease. Front Neurosci. 2014; 8 (OCT): 1–23.
4. Thies W, Bleiler L. 2011 Alzheimer's disease facts and figures. Alzheimers Dement. 2011; 7 (2): 208–44.
5. Tackenberg C, Kulic L, Nitsch RM. Familial Alzheimer's disease mutations at position 22 of the amyloid β-peptide sequence differentially affect synaptic loss, tau phosphorylation and neuronal cell death in an ex vivo system. PLoS One. 2020; 15 (9): 1–14.
6. Hayne DJ, Lim S, Donnelly PS. Metal complexes designed to bind to amyloid-β for the diagnosis and treatment of Alzheimer's disease. Chem Soc Rev. 2014; 43 (19): 6701–15.
7. Carroll CM, Li YM. Physiological and pathological roles of the γ-secretase complex. Brain Res Bull. 2016; 126: 199–206.
8. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat Rev Mol Cell Biol. 2007; 8 (2): 101–12.
9. Citron M. Alzheimer's disease: strategies for disease modification. Nat Rev Drug Discov. 2010; 9 (5): 387–98.
10. Masters CL, Simms G, Weinman NA, et al. Molecular mechanisms for Alzheimer's disease: implications for neuroimaging and therapeutics. J Neurochem. 2006; 97 (6): 1700–25.
11. Masters CL, Beyreuther K. Science, medicine, and the future: Alzheimer's disease. BMJ. 1998; 316 (7129): 446.
12. Hippius H, Neundörfer G. The discovery of Alzheimer's disease. Dialogues Clin Neurosci. 2003; 5 (1): 101–8.
13. Zolotarev YA, Dadayan AK, Bocharov EV, et al. Pharmacokinetics and molecular modeling indicate nAChRα4-derived peptide HAEE goes through the blood-brain barrier. Biomolecules. 2021; 11 (6): 902.
14. Maack T, Johnson V, Kau ST, et al. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. Kidney Int. 1979; 16 (3): 251–70.
15. Agu RU, Ugwoke MI, Armand M, et al. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir Res. 2001; 2 (4): 198–209.

Литература

1. Huang LK, Kuo HY, Chen HJ, et al. Clinical trials of new drugs for Alzheimer disease: a 2020–2023 update. J Dent Res. 2023; 88 (3): 1–19.
2. Facts D. Alzheimer's disease facts and figures report: executive summary. Alzheimers Dement. 2024; 20 (5): 40–42.
3. Adlard PA, Bush AI. A review of β-amyloid neuroimaging in Alzheimer's disease. Front Neurosci. 2014; 8 (OCT): 1–23.
4. Thies W, Bleiler L. 2011 Alzheimer's disease facts and figures. Alzheimers Dement. 2011; 7 (2): 208–44.
5. Tackenberg C, Kulic L, Nitsch RM. Familial Alzheimer's disease mutations at position 22 of the amyloid β-peptide sequence differentially affect synaptic loss, tau phosphorylation and neuronal cell death in an ex vivo system. PLoS One. 2020; 15 (9): 1–14.
6. Hayne DJ, Lim S, Donnelly PS. Metal complexes designed to bind to amyloid-β for the diagnosis and treatment of Alzheimer's disease. Chem Soc Rev. 2014; 43 (19): 6701–15.
7. Carroll CM, Li YM. Physiological and pathological roles of the γ-secretase complex. Brain Res Bull. 2016; 126: 199–206.
8. Haass C, Selkoe DJ. Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide. Nat Rev Mol Cell Biol. 2007; 8 (2): 101–12.
9. Citron M. Alzheimer's disease: strategies for disease modification. Nat Rev Drug Discov. 2010; 9 (5): 387–98.
10. Masters CL, Simms G, Weinman NA, et al. Molecular mechanisms for Alzheimer's disease: implications for neuroimaging and therapeutics. J Neurochem. 2006; 97 (6): 1700–25.
11. Masters CL, Beyreuther K. Science, medicine, and the future: Alzheimer's disease. BMJ. 1998; 316 (7129): 446.
12. Hippius H, Neundörfer G. The discovery of Alzheimer's disease. Dialogues Clin Neurosci. 2003; 5 (1): 101–8.
13. Zolotarev YA, Dadayan AK, Bocharov EV, et al. Pharmacokinetics and molecular modeling indicate nAChRα4-derived peptide HAEE goes through the blood-brain barrier. Biomolecules. 2021; 11 (6): 902.
14. Maack T, Johnson V, Kau ST, et al. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. Kidney Int. 1979; 16 (3): 251–70.
15. Agu RU, Ugwoke MI, Armand M, et al. The lung as a route for systemic delivery of therapeutic proteins and peptides. Respir Res. 2001; 2 (4): 198–209.

BRAIN NATRIURETIC PEPTIDE AND CORTICOSTERONE DYNAMICS IN EXPERIMENTAL CHRONIC HEART FAILURE DURING PHYSICAL ACTIVITY

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Moderate exercise not only has a positive impact on overall health, but also can serve as a rather accessible preventive measure for maintaining health, particularly the cardiovascular system health. The study aimed to assess the cardiovascular system adaptive capacity in chronic heart failure with moderate exercise in different age groups. Moderate exercise was induced in 6- and 19-month-old rats by forced swimming in a water bath at 32–34°C. During training, chronic heart failure was induced by intraperitoneal administration of the anthracycline antibiotic doxorubicin (Teva) at a cumulative dose of 15 mg/kg, divided into 6 injections over 14 days. Serum levels of brain natriuretic peptide and corticosterone were determined by ELISA every seven days throughout the experiment in all rats. It was found that with chronic heart failure and moderate exercise, myocardial adaptation was significantly higher in both age groups. It was most pronounced in aging rats, as evidenced by the dynamic changes of serum natriuretic peptide levels throughout the experiment. In both fertile-age and aging rats, the body's adaptive capacity in the event of cardiac dysfunction with moderate exercise is higher than in the absence of training.

Keywords: physical exercise, chronic heart failure, brain natriuretic peptide, corticosterone

Author contribution: Dzhandarova TI — experimental design and procedure, material resources, editing and data analysis; Tabunshchikova MO — animal handling and enzyme-linked immunoassay, statistical processing and data analysis; Kubanov SI — design and data analysis; Domenyuk DA — material resources for the study.

Compliance with ethical standards: the study was approved by the Ethics Committee of the Stavropol State Medical University (protocol No. 100 dated 17 June 2021) and conducted in accordance with the requirements of the Order of the Ministry of Health of the Russian Federation No. 708n of 23.08.2010 "On approval of principles of laboratory practice", Orders of the Ministry of Health of the USSR No. 742 of 13.11.1984 "On approval of the experimental animal handling principles" and No. 48 of 23.01.1985 "On regulation of the use of experimental animals".

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ДИНАМИКА МОЗГОВОГО НАТРИЙУРЕТИЧЕСКОГО ПЕПТИДА И КОРТИКОСТЕРОНА ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ХРОНИЧЕСКОЙ СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТИ НА ФОНЕ ФИЗИЧЕСКИХ НАГРУЗОК

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Умеренные физические нагрузки не только оказывают положительное влияние на общее состояние организма, но и могут выступать в качестве вполне доступного профилактического средства для сохранения здоровья, в частности, сердечно-сосудистой системы. Целью исследования было оценить адаптационные возможности сердечно-сосудистой системы при хронической сердечной недостаточности на фоне умеренных физических нагрузок в разных возрастных категориях. Умеренные физические нагрузки создавали у крыс в возрасте 6 и 19 месяцев принудительным плаванием в ванне с водой температурой 32–34 °С. На фоне тренировок хроническую сердечную недостаточность вызывали путем внутрибрюшинного введения антрациклинового антибиотика доксорубицин в кумулятивной дозе 15 мг/кг, разделенной на шесть инъекций в течение 14 дней. У всех крыс на протяжении эксперимента через каждые семь дней определяли в сыворотке крови содержание мозгового натрийуретического пептида и кортикостерона иммуноферментным методом. Установлено, что при хронической сердечной недостаточности на фоне проводимых умеренных нагрузок адаптация миокарда значимо выше в обеих возрастных группах. Наиболее ярко она проявляется у стареющих крыс, о чем свидетельствует динамика содержания натрийуретического пептида в сыворотке крови на протяжении всего эксперимента. Как у крыс репродуктивного периода, так и у стареющих крыс адаптационные возможности организма при возникновении нарушений сердечной деятельности на фоне умеренных физических нагрузок оказываются выше, чем в условиях отсутствия тренировок.

Ключевые слова: физические нагрузки, хроническая сердечная недостаточность, мозговой натрийуретический пептид, кортикостерон

Вклад авторов: Т. И. Джандарова — дизайн и проведение эксперимента, материальная база, редактирование и анализ данных; М. О. Табунщикова — работа с животными и проведение иммуноферментного анализа, статистическая обработка и анализ данных; С. И. Кубанов — дизайн и анализ данных; Д. А. Доменюк — материальная база исследования.

Соблюдение этических стандартов: исследование одобрено локальным этическим комитетом ФГБОУ ВО Ставропольский государственный медицинский университет Минздрава России (протокол № 100 от 17 июня 2021 г.), проведено в соответствии с требованиями Приказа МЗ и СР РФ №708н от 23.08.2010 «Об утверждении правил лабораторной практики», требованиями Приказа МЗ СССР № 742 от 13.11.1984 «Об утверждении правил проведения работ с использованием экспериментальных животных» и № 48 от 23.01.1985 «О контроле за проведением работ с использованием экспериментальных животных».

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It is well known that physical exercise has a positive effect on the overall body condition and can be a rather accessible preventive measure for maintaining health, particularly the cardiovascular system health.

A number of studies show the effectiveness of rehabilitation programs, in which exercise is included, based on the dynamic changes in the brain natriuretic peptide levels in healthy individuals. With the existing cardiovascular disorders, physical exercise causes a dramatic brain natriuretic peptide level increase, which can be associated with aggravation of symptoms [1, 2]. Furthermore, physical exercise is also recommended as pre-habilitation for patients, who are to undergo cardiovascular surgery aimed to improve functional capacity of the heart [3].

At the same time, there is currently no consensus about what exercises and for how long to do these to improve cardiac activity [4], and the mechanisms underlying the effects of physical exertion of varying intensity on the cardiovascular system are currently poorly understood [5–7], especially in elderly people.

Along with other signs, the age-related changes associated with natural ageing are accompanied by the major shift of the cardiovascular system neuroendocrine regulation. In particular, there are considerable functional changes in the hypothalamic-pituitary-adrenal axis. The emerging neuroendocrine dysregulation of the cardiovascular system results from the increase in basal adrenocorticotropic hormone (ACTH) and cortisol levels. Furthermore, inadequate elevation of the levels of these hormones is associated with physical stress. Such alterations result immediately in the increased risk of cardiovascular disorders, even during the short-term treatment with low-dose glucocorticoids, which affects future life, prognosis, and outcome of the underlying disease. The increase in the levels of these hormones poses a greater risk of carsiovascular events, such as myocardial infarction, stroke, coronary artery disease, chronic heart failure [8, 9]. Undoubtedly, any body's process leading to changes in the cardiovascular system activates the endocrine function of the myocardium. Atrial cardiomyocytes produce natriuretic peptides referred to as the atrial natriuretic factor (or atrial natriuretic peptide) and brain natriuretic peptide (or B-type natriuretic peptide). Natriuretic peptides that have many physiological effects are involved in numerous pathophysiological processes. Furthermore, plasma levels of natriuretic peptides, specifically brain natriuretic peptide, represent potent diagnostic and prognostic biomarkers of heart diseases [10–12].

The study aimed to assess the cardiovascular system adaptive capacity in chronic heart failure with moderate exercise in different age groups.

METHODS

The study involved 72 male Wistar rats aged 6 months (fertile age) and 19 months (senescent rats). The animals kept in natural light at the temperature of 20–22 °C and relative humidity of 60–70% had ad libitum access to drinking water and standard vivarium feed.

Animals of each age group were divided into four groups: the first group included intact rats (controls); the second one included rats getting moderate exercise; the third one included rats, in which chronic heart failure was simulated; the fourth one included rats, in which chronic heart failure was simulated with moderate exercise.

Chronic heart failure was simulated in rats by intraperitoneal administration of the cardiotoxic dose of the anthracycline antibiotic doxorubicin (Teva) at a cumulative dose of 15 mg/kg, divided into 6 injections over 14 days [13].

The controls for intraperitoneal administration we represented by the group of animals receiving intraperitoneal injections of saline. The analysis and comparison of the data obtained with the values of intact animals revealed no significant differences. That is why in the study the data of experimental animals were compared with the values of intact control animals.

Moderate exercise was induced in the animals by forced swimming in a water bath at 32–34 °C. Training was conducted daily, five days a week throughout three weeks. The cycle consisted of 15 days with training sessions. The first training session lasted for 2 min; the duration of each subsequent session to the day 21 was increased by 4 min. The animals were included in the experiment after 1, 2, and 3 weeks of training. In all rats, 1 mL of blood was collected from the tail vein into the test tubes containing clotting activator under anesthesia in due course. Blood was centrifuged at 3000 rpm for 15 min, then collected and stored at –35 °C.

Brain natriuretic peptide (NT-proBNP) was determined by sandwich ELISA using the NTBNP-ELISA-BEST kit, and corticosterone was determined using the DRG reagent kit (DRG Instruments GmbH, Germany). Optical density of the studied hormones was measured using the plate spectrophotometer equipped with the cell holder combined with the software and μDrop plate, Thermo for microvolume analysis (Multiskan SkyHigh, USA). Significance of differences in the studied indicators was determined using the Student's t-test, when the sample distribution was normal. The distribution was tested for normality using the Shapiro–Wilk test. The results were considered significant at $p < 0.05$.

RESULTS

The fertile age rats showed no differences in serum brain natriuretic peptide levels from both baseline values and values of controls within the first two weeks of training. During adaptation to training a significant decrease in serum levels of the peptide relative to the data of control rats was reported in these animals (Fig. 1).

In the group of rats of the same age, in which chronic heart failure was simulated, the considerably high serum brain natriuretic peptide levels relative to both baseline and the data of control rats were observed throughout the experiment (Fig. 1).

When modeling chronic heart failure with moderate exercise, a rather high brain natriuretic peptide concentration in blood serum was revealed in the first week of training, and in subsequent weeks of training the brain natriuretic peptide levels significantly decreased compared to the data reported for rats with chronic heart failure (Fig. 1). The changes identified suggest that with moderate exercise myocardial adaptation to possible cardiac dysfunction is significantly higher.

During adaptation to moderate exercise, a significant decrease in serum corticosterone levels relative to both baseline and values of control rats was revealed in fertile age rats in the second and third weeks of the experiment (Fig. 2). In the fertile age rats, in which chronic heart failure was simulated, serum corticosterone levels were significantly high relative to both baseline values of the same rats and the values of control rats in the beginning of the experiment. Later the adaptation processes decreased considerably in these animals, as suggested by significantly low serum corticosterone levels in the end of the third week of the study (Fig. 2).

When modeling chronic heart failure in fertile rats with training, there was a significant increase in serum corticosterone

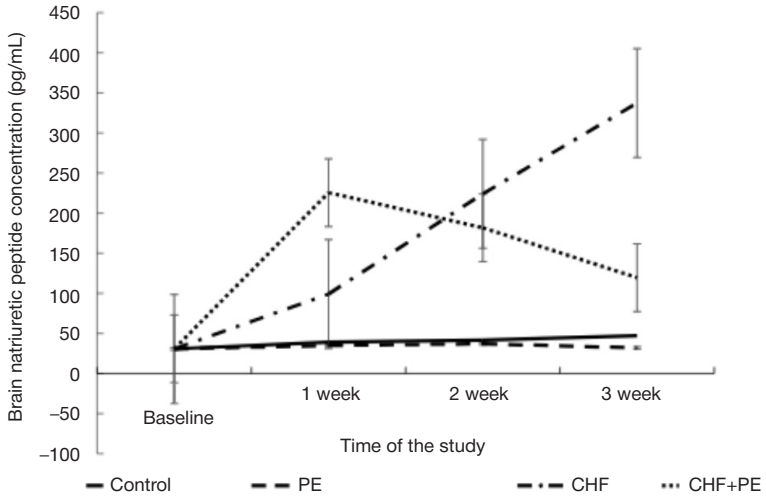

Fig. 1. Serum concentration of brain natriuretic peptide in 6-month-old rats

levels relative to baseline and the data of control rats in the beginning of the experiment. Later during the experiment serum corticosterone levels decreased to the levels of control rats, and in the end of the third week of the study serum corticosterone levels were significantly lower compared to the data of control rats, but higher, than in rats with chronic heart failure (Fig. 2). Therefore, adaptive capacity of the fertile age animals developing cardiac disorders with moderate exercise still turn out to be higher, than under conditions of no training.

As demonstrated by further findings, in the aging rats, regular moderate exercise starting from the first week of the experiment contributed to the significant decrease in serum brain natriuretic peptide levels compared to both baseline data of the same rats and the values of control rats (Fig. 3). Considering the age-related increase in the levels of this peptide in aging rats, it can be assumed that moderate exercise under conditions of no cardiovascular system dysfunction has a beneficial effect on the state of the myocardium.

In the group of aging rats, in which chronic heart failure was simulated, a significant increase in the serum levels of brain natriuretic peptide relative to the baseline values of the same animals and the values of control rats was reported throughout the experiment (Fig. 3).

When modeling chronic heart failure with moderate exercise in aging rats, the significantly high serum brain natriuretic peptide levels compared to both baseline and the values of control rats were revealed. However, after the first week of training the peptide levels decreased significantly compared to the data of rats with chronic heart failure (Fig. 3). The changes revealed suggest that regular training improves cardiovascular system function and heart functioning effectiveness in the aging body even in cases of developing cardiac dysfunction.

During adaptation to regular training the aging rats showed a significant moderate increase in serum corticosterone levels compared to both baseline corticosterone levels and the values of control rats throughout the experiment (Fig. 4). In the aging rats, in which chronic heart failure was simulated, serum corticosterone levels were significantly high relative to both baseline values of the same rats and the values of control rats in the first week of the experiment. In the next weeks of the experiment, especially in the end of the third week, these rats showed a significant decrease in serum corticosterone levels, which suggests lower adaptation against the background of cardiac dysfunction (Fig. 4).

When modeling chronic heart failure in aging rats with training, the significant increase in serum corticosterone levels compared to both baseline values and the data of control rats was reported in the beginning of the experiment. In the remaining time of the experiment, up to the end of the third week, the decrease in serum corticosterone levels to the values of control rats took place (Fig. 4). Therefore, when the aging rats develop cardiac dysfunction with moderate exercise,

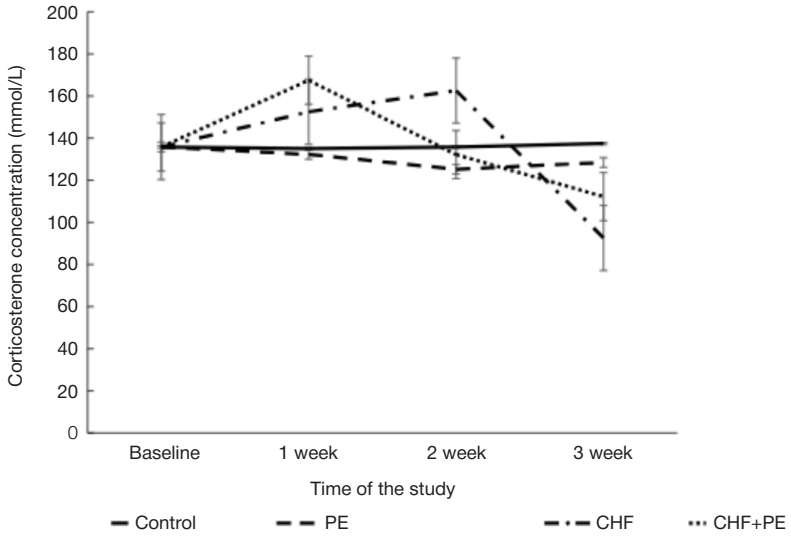

Fig. 2. Serum concentration of corticosteroids in 6-month-old rats

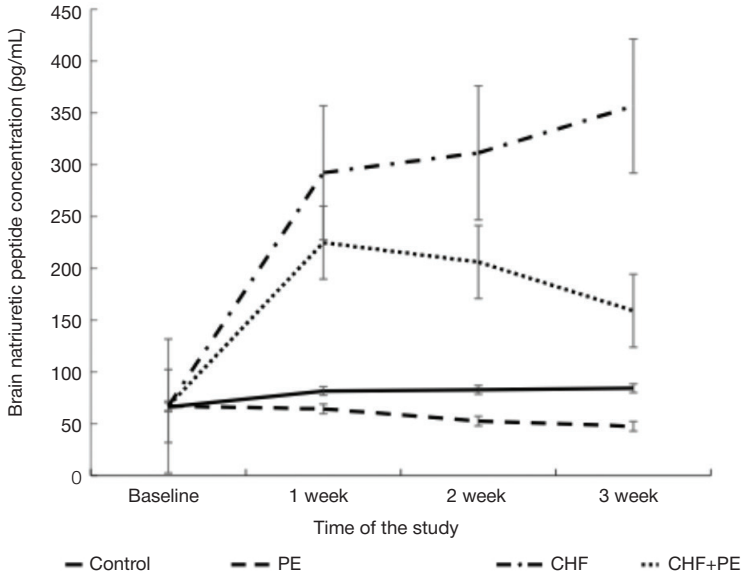

Fig. 3. Serum concentration of brain natriuretic peptide in 19-month-old rats

body's adaptive capacity turns out to be higher, than under conditions of no training.

DISCUSSION

As is well known, considerable changes in all body's system occur during aging, which results in limitation of the capability of adaptation to environmental factors and depletion of energy resources. At the same time, certainly, pathophysiological mechanisms are activated that finally lead to the development of cardiovascular and other disorders. All the above contributes to limitation of motor activity of an elderly individual, which also can enhance the increase in destructive processes in the body.

Today, the family of cardiac hormones, such as brain natriuretic peptide, the levels of which are increased with ventricular wall dilatation caused by overload or dysfunction, represent one of the widely used markers of the cardiac function status. Brain natriuretic peptide is an important biomarker for the diagnosis and monitoring of heart failure, since the increase in blood concentration of the peptide is correlated to the heart failure severity [1, 2, 10].

As is well known, heart failure results from structural and functional cardiac impairment. It is characterized by water and sodium retention, electrolyte imbalance, and kidney function deterioration. Our previous studies also showed that the experimental chronic heart failure led to the progressive increase in serum levels of aldosterone, sodium, and potassium [14]. The effect of brain natriuretic peptide is to reduce the load on the myocardium through enhancement of diuresis, increased sodium excretion. The elevated blood brain natriuretic peptide concentration indicates the decreased heart muscle contractility and allows one to judge about heart failure even without echocardiography. The main stimulus for natriuretic peptide secretion are the increase in myocardial tension with increasing blood pressure or volume in the left heart ventricle and mechanical stretching of the atria [15]. Glucocorticoids, in turn, have a multifaceted effect on the myocardium due to metabolic alterations, contribute to blood pressure increase, which represents and independent risk factor of many cardiovascular disorders [8].

The feature of our study is that we modeled moderate exercise before the emergence of any significant disorder of the cardiovascular system in the fertile period of ontogeny and during aging of the body. It was found that adaptation to moderate physical exercise was associated with the decrease in serum brain natriuretic peptide levels in both age groups.

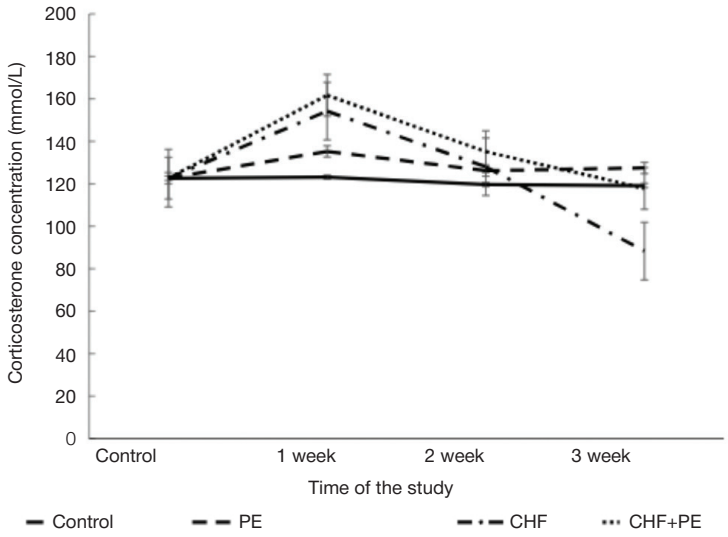

Fig. 4. Serum concentration of corticosteroids in 19-month-old rats

When creating the model of chronic heart failure with moderate exercise, adaptation of the myocardium to possible cardiac dysfunction increases considerably in both age groups, which is particularly evident in aging rats. Furthermore, in both fertile age and aging rats, body's adaptive capacity is higher with moderate exercise, than under conditions of no training, which is indicated by the dynamic changes in serum corticosterone levels throughout the experiment. Positive impact of moderate exercise on body's physiological processes has been determined by other factors. These papers report that physical activity is the source of substances possessing therapeutic effects that are produced by the body itself. Regular physical activity has a beneficial effect on the tissue homeostasis, function, and interaction [5].

However, the long-term heavy physical exertion can adversely affect the condition of cardiomyocytes, as indicated by the significantly elevated brain natriuretic peptide binding fatty acids, copeptin, troponin [16, 17]. Undoubtedly, physical exercise has a multifaceted effect on the brain natriuretic peptide levels depending on the exertion intensity and duration, as well as on the baseline cardiovascular system state [1]. As also shown by our studies, regular moderate exercise should have its place in life since young adulthood, before the emergence of the age-related cardiovascular system alterations. It will make it possible to improve the aging body's quality of life.

CONCLUSIONS

It has been found that when creating the model of chronic heart failure with moderate exercise, adaptation of the myocardium to possible cardiac dysfunction increases significantly in both age groups, which is particularly evident in aging rats. In both fertile age and aging rats, body's adaptive capacity in cases of developing cardiac dysfunction turns out to be higher with moderate exercise, than under conditions of no training, as indicated by the dynamic changes in serum corticosterone levels throughout the experiment.

References

1. Meshtel AV, Miroshnikov AB, Smolenskij AV. Vlijanie fizicheskoj nagruzki na mozgovoj natrijureticheskij peptid pri razlichnyh sostojanijah serdechno-sosudistoj sistemy: obzor predmetnogo polja. Medicinskij alfavit. 2024; (16): 65–68. Available from: https://doi.org/10.33667/2078-5631-2024-16-65-68.
2. Badiani S, van Zalen J, Althunayyan A, Al-Borikan S, Treibel T, Marshall A., et al. Natriuretic peptide release during exercise in patients with valvular heart disease: A systematic review. Int J Clin Pract. 2021; 75 (10): 14137. Available from: https://doi.org/10.1111/ijcp.14137.
3. Drudi LM, Tat J, Ades M, Mata J, Landry T, MacKenzie KS, et al. Preoperative Exercise Rehabilitation in Cardiac and Vascular Interventions. Journal of Surgical Research. 2019; 237: 3–11. Available from: https://doi.org/10.1016/j.jss.2018.11.042.
4. Alphonsus CS, Govender P, Rodseth RN, Biccard BM. The role of cardiac rehabilitation using exercise to decrease natriuretic peptide levels in non-surgical patients: a systematic review. Perioper Med (Lond). 2019; 18: 8–14. Available from: https://doi.org/10.1016/j.jss.2018.11.042.
5. Kotovskaja JuV, Tkachjova ON, Runihina NK, Luzina AV. Fizicheskie nagruzki kak sredstvo profilaktiki serdechno-sosudistyh zabolevanij u pozhilyh pacientov. Doktor.Ru. 2019; 2(157): 19–22. DOI: 10.31550/1727-2378-2019-157-2-19-22. Russian.
6. Nikulin JuI. Vlijanie atleticheskoj gimnastiki na adaptaciju serdechno-sosudistoj sistemy studentov k fizicheskim nagruzkam. Uchenye zapiski universiteta im. P.F. Lesgafta. 2021; 1 (191): 271–4. Russian.
7. Bajseitova AB, Moldabekov EO, Amanbaeva GT. Vlijanie fizicheskih uprazhnenij na serdechno sosudistuju sistemu. Innovacii. Nauka. Obrazovanie. 2022; 52: 900–8. Russian.
8. Dzherieva IS, Volkova NI, Davidenko IJu, Reshetnikov IB, Brovkina SS, Avakova SM, Tishhenko JuV. Gljukokortikoidnaja terapija — faktor riska serdechno-sosudistyh zabolevanij. Medicinskij vestnik Juga Rossii. 2022;13(3): 93–106. https://doi.org/10.21886/2219-8075-2022-13-3-93-106. Russian.
9. Kosharnaja RS, Belaja ZhE, Mamedova EO, i dr. Serdechnaja nedostatochnost' pri jendogennom giperkorticizme: osobennosti diagnostiki i obratimost' pri dostizhenii remissii zabolevanija. Jeffektivnaja farmakoterapija. 2023; 19 (55): 6–14. DOI: 10.33978/2307-3586-2023-19-55-6-14.
10. Götze JP, Bruno BG, Ramos HR, et al. Cardiac natriuretic peptides. Nat Rev Cardiol. 2020; 17: 698–717. Available from: https://doi.org/10.1038/s41569-020-0381-0.
11. Bugrova ML, Jakovleva EI, Abrosimov DA. Vzaimosvjaz' intensivnosti sinteza, nakoplenija i sekrecii predserdnogo natrijureticheskogo peptida kardiomiocitov s urovnem reguljacii serdechnogo ritma u krys v uslovijah rannego postreperfuzionnogo perioda. Sovremennye tehnologii v medicine. 2012; 3: 26–30. Russian.
12. Bugrova ML, Abrosimov DA, Jakovleva EI. Vlijanie Meksidola na mozgovoj natrijureticheskij peptid kardiomiocitov v postreperfuzionnom periode v jeksperimente. Sovremennye tehnologii v medicine. 2015; 7 (3): 40–46. Russian.
13. Kazachenko AA, Okovityj SV, Kulikov AN, i dr. Jeksperimental'noe modelirovanie hronicheskoj serdechnoj nedostatochnosti. Biomedicina. 2013; 3: 41–48. Russian.
14. Dzhandarova TI, Tabunshhikova MO, Kubanov SI, Manveljan JeA, Jebzeeva LH. Jelektrolitnyj disbalans pri narushenii dejatel'nosti serdechno-sosudistoj sistemy i ego profilaktika. Sovremennye voprosy biomediciny. 2024; 8 (27): 7–8. Russian.
15. Kontorshhikova KN. Kliniko-informacionnaja cennost' novejshih biomarkerov. Remedium Privolzh'e. 2015; 4 (134): 30–33. Russian.
16. Perrone MA, Macrini M, Maregnani A, et al. The effects of a 50 km ultramarathon race on high sensitivity cardiac troponin I and NT-proBNP in highly trained athletes [published online ahead of print, 2020 Jul 10]. Minerva Cardioangiol. 2020; 68 (4): 305–12. Availale from: https://doi.org/10.23736/S0026-4725.20.05281-0.
17. Martmez-Navarro I, Sanchez-Gomez JM, Collado-Boira EJ, et al. Cardiac Damage Biomarkers and Heart Rate Variability Following a 118-Km Mountain Race: Relationship with Performance and Recovery. J Sports Sci Med. 2019; 18 (4): 615–22.

Литература

1. Мештель А. В., Мирошников А. Б., Смоленский А. В. Влияние физической нагрузки на мозговой натрийуретический пептид при различных состояниях сердечно-сосудистой системы: обзор предметного поля. Медицинский алфавит. 2024; (16): 65–68. Доступно по ссылке: https://doi.org/10.33667/2078-5631-2024-16-65-68.
2. Badiani S, van Zalen J, Althunayyan A, Al-Borikan S, Treibel T, Marshall A., et al. Natriuretic peptide release during exercise in patients with valvular heart disease: A systematic review. Int J Clin Pract. 2021; 75 (10): 14137. Available from: https://doi.org/10.1111/ijcp.14137.
3. Drudi LM, Tat J, Ades M, Mata J, Landry T, MacKenzie KS, et al. Preoperative Exercise Rehabilitation in Cardiac and Vascular

Interventions. Journal of Surgical Research. 2019; 237: 3–11. Available from: https://doi.org/10.1016/j.jss.2018.11.042.
4. Alphonsus CS, Govender P, Rodseth RN, Biccard BM. The role of cardiac rehabilitation using exercise to decrease natriuretic peptide levels in non-surgical patients: a systematic review. Perioper Med (Lond). 2019; 18: 8–14. Available from: https://doi.org/10.1016/j.jss.2018.11.042.
5. Котовская Ю. В., Ткачёва О. Н., Рунихина Н. К., Лузина А. В. Физические нагрузки как средство профилактики сердечно-сосудистых заболеваний у пожилых пациентов. Доктор.Ру. 2019; 2 (157): 19–22. DOI: 10.31550/1727-2378-2019-157-2-19-22.
6. Никулин Ю. И. Влияние атлетической гимнастики на адаптацию сердечно-сосудистой системы студентов к физическим нагрузкам. Ученые записки университета им. П. Ф. Лесгафта. 2021; 1 (191): 271–4.
7. Байсеитова А. Б., Молдабеков Е. О., Аманбаева Г. Т. Влияние физических упражнений на сердечно-сосудистую систему. Инновации. Наука. Образование. 2022; 52: 900–8.
8. Джериева И. С., Волкова Н. И., Давиденко И. Ю., Решетников И. Б., Бровкина С. С. и др. Глюкокортикоидная терапия — фактор риска сердечно-сосудистых заболеваний. Медицинский вестник Юга России. 2022; 13 (3): 93–106. Доступно по ссылке: https://doi.org/10.21886/2219-8075-2022-13-3-93-106.
9. Кошарная Р. С., Белая Ж. Е., Мамедова Е. О. и др. Сердечная недостаточность при эндогенном гиперкортицизме: особенности диагностики и обратимость при достижении ремиссии заболевания. Эффективная фармакотерапия. 2023; 19 (55): 6–14. DOI 10.33978/2307-3586-2023-19-55-6-14.
10. Götze JP, Bruno BG, Ramos HR, et al. Cardiac natriuretic peptides. Nat Rev Cardiol. 2020; 17: 698–717. Available from: https://doi.org/10.1038/s41569-020-0381-0.
11. Бугрова М. Л., Яковлева Е. И., Абросимов Д. А. Взаимосвязь интенсивности синтеза, накопления и секреции предсердного натрийуретического пептида кардиомиоцитов с уровнем регуляции сердечного ритма у крыс в условиях раннего постреперфузионного периода. Современные технологии в медицине. 2012; 3: 26–30.
12. Бугрова М. Л., Абросимов Д. А., Яковлева Е. И. Влияние Мексидола на мозговой натрийуретический пептид кардиомиоцитов в постреперфузионном периоде в эксперименте. Современные технологии в медицине. 2015; 7 (3): 40–46.
13. Казаченко А. А., Оковитый С. В., Куликов А. Н. и др. Экспериментальное моделирование хронической сердечной недостаточности. Биомедицина. 2013; 3: 41–48.
14. Джандарова Т. И., Табунщикова М. О., Кубанов С. И., Манвелян Э. А., Эбзеева Л. Х. Электролитный дисбаланс при нарушении деятельности сердечно-сосудистой системы и его профилактика. Современные вопросы биомедицины. 2024; 8 (27): 7–8.
15. Конторщикова К. Н. Клинико-информационная ценность новейших биомаркеров. Ремедиум Приволжье. 2015; 4 (134): 30–33.
16. Perrone MA, Macrini M, Maregnani A, et al. The effects of a 50 km ultramarathon race on high sensitivity cardiac troponin I and NT-proBNP in highly trained athletes [published online ahead of print, 2020 Jul 10]. Minerva Cardioangiol. 2020; 68 (4): 305–12. Availale from: https://doi.org/10.23736/S0026-4725.20.05281-0.
17. Martmez-Navarro I, Sanchez-Gomez JM, Collado-Boira EJ, et al. Cardiac Damage Biomarkers and Heart Rate Variability Following a 118-Km Mountain Race: Relationship with Performance and Recovery. J Sports Sci Med. 2019; 18 (4): 615–22.