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CHARACTERISTICS OF THE INTESTINAL MICROBIOME IN STROKE PATIENTS WITH INSULIN RESISTANCE

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Abstract

Introduction. Advances have been made in the treatment and prevention of stroke in recent decades, but its burden remains high. New targets are needed to improve outcomes. Recent studies highlight the role of the intestinal microbiota in the pathogenesis of stroke. Changes in the composition of the microbiota, known as dysbiosis, are associated with risk factors for stroke such as obesity, metabolic disorders and atherosclerosis. In acute cerebral ischemia, the intestinal microbiota affects the interactions between the intestine and the brain, forming the microbiota- intestine -brain axis. Dysbiosis before a stroke affects its outcomes. Clinical studies show that in acute ischemic stroke, dysbiosis is associated with metabolism, inflammation, and functional outcomes. Modulation of the microbiota or its metabolites improves conditions associated with the pathogenesis of stroke, including inflammation, cardiometabolic diseases, atherosclerosis, and thrombosis.

Aim: to study the characteristics of the intestinal microbiome in patients with stroke and insulin resistance.

Materials and methods: observational, analytical, and cross-sectional methods, taxonomic analysis of the intestinal microbiota, and NGS sequencing.

Results. A species analysis of the microbiota in patients with stroke and IR revealed a correlation with lactate-producing bacteria Streptococcus, butyrate- and acetate-producing bacteria Bacteroides (dorei, massiliensis, plebeius, tobetsuensis) and Dialester invisus, a propionate producer. The presence of such poorly understood predictors of stroke in patients with insulin resistance suggests a possible influence of the intestinal microbiota in maintaining inflammation, blood pressure formation, and stroke in patients with insulin resistance. The role of bacteria producing acetate, butyrates, and propionates in the active fermentation of starches, affecting triglycerides with a subsequent increase in insulin resistance is shown. Host-microbiota interactions involving inflammatory and metabolic pathways appear to play a role in the occurrence of cardiovascular diseases. Lactate-, butyrate-, and propionate producing bacteria are of no small importance in the pathogenesis of hypertension with IR**.**

Conclusions. The obtained results underscore a potential link between dysbiosis associated with stroke and the balance of organic acids produced by intestinal bacteria, specifically an inverse relationship between stroke and the levels of acetate and butyrate. One contributing factor is intestinal inflammation related to dysbiosis, which helps maintain high blood pressure.

Keywords**:** *microbiome, insulin resistance, arterial hypertension, stroke.*

ХАРАКТЕРИСТИКА МИКРОБИОМА КИШЕЧНИКА ПРИ ИНСУЛЬТЕ У ПАЦИЕНТОВ С ИНСУЛИНОРЕЗИСТЕНТНОСТЬЮ

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Резюме

Введение. За последние десятилетия достигнуты успехи в лечении и профилактике инсульта, однако его бремя остается высоким. Требуются новые мишени для улучшения исходов. Недавние исследования выделяют роль кишечной микробиоты в патогенезе инсульта. Изменения в составе микробиоты, известные как дисбактериоз, связаны с факторами риска инсульта — ожирением, метаболическими нарушениями и атеросклерозом. При острой ишемии головного мозга кишечная микробиота влияет на взаимодействия между кишечником и мозгом, образуя ось микробиота-кишечник-мозг. Дисбактериоз до инсульта влияет на его исходы. Клинические исследования показывают, что при остром ишемическом инсульте дисбиоз связан с метаболизмом, воспалением и функциональными исходами. Модуляция микробиоты или её метаболитов улучшает состояния, связанные с патогенезом инсульта, включая воспаление, кардиометаболические заболевания, атеросклероз и тромбоз.

Цель: изучить характеристику микробиома кишечника у пациентов с инсультом при инсулинорезистентности.

Материалы и методы: обсервационный, аналитический и перекрестный методы, таксономический анализ кишечной микробиоты и метод NGS секвенирования.

Результаты. Видовой анализ микробиоты у пациентов с инсультом и ИР выявил корреляцию с лактат-продуцирующими бактериями Streptococcus, бутират- и ацетат-продуцирующих бактерий Bacteroides (dorei, massiliensis, plebeius, tobetsuensis) и Dialester invisus продуцент пропионата. Наличие таких малоизученных предикторов инсульта у пациентов с инсулинорезистентностью, говорят о возможном влиянии микробиоты кишечника в поддержании воспаления, формирования артериального давления и инсульта у пациентов с инсулинорезистентностью. Показана роль бактерии продуцента ацетата, бутиратов, пропионатов при активной ферментации крахмалов, влияющая на триглицериды с последующим увеличением инсулинорезистентности. Взаимодействия хозяин-микробиота, включающие воспалительные и метаболические пути, по-видимому, играют роль в возникновении сердечно-сосудистых заболеваний. Немаловажное значение в патогенезе при возникновении АГ с ИР имеют лактат-, бутират- и пропионат – продуцирующие бактерии.

Выводы. Полученные результаты подчеркнули возможную связь дисбиоза, ассоциированного с инсультом, с балансом органических кислот, производимых кишечными бактериями, а именно — обратную связь инсульта с уровнем ацетата и бутирата. Одной из причин является кишечное воспаление, связанное с дисбактериозом, и способствует поддержанию высокого артериального давления.

Ключевые слова: *микробиом, инсулинорезистентность, артериальная гипертония, инсульт.*

Түйіндеме

ИНСУЛИНГЕ ТӨЗІМДІЛІГІ БАР НАУҚАСТАРДА ИНСУЛЬТ КЕЗІНДЕ ІШЕК МИКРОБИОМАСЫНЫҢ СИПАТТАМАСЫ

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Кіріспе. Соңғы онжылдықтарда инсультті емдеуде және алдын алуда жетістіктерге қол жеткізілді, бірақ оның ауыртпалығы жоғары болып қала береді. Нәтижелерді жақсарту үшін жаңа мақсаттар қажет. Соңғы зерттеулер инсульт патогенезіндегі ішек микробиотасының рөлін көрсетеді. Дисбиоз деп аталатын микробиотаның құрамындағы өзгерістер инсульттің қауіп факторларымен байланысты — семіздік, метаболикалық бұзылулар және атеросклероз. Жедел ми ишемиясында ішек микробиотасы ішек пен мидың өзара әрекеттесуіне әсер етіп, микробиота-ішек-ми осін құрайды. Инсультке дейінгі дисбиоз оның нәтижелеріне әсер етеді. Клиникалық зерттеулер жедел ишемиялық инсульт кезінде дисбиоз метаболизммен, қабынумен және функционалдық нәтижелермен байланысты екенін көрсетеді. Микробиотаны немесе оның метаболиттерін модуляциялау инсульт патогенезіне байланысты жағдайларды жақсартады, соның ішінде қабыну, кардиометаболикалық аурулар, атеросклероз және тромбоз.

Мақсаты: инсулинге төзімділігі бар инсультпен ауыратын науқастарда ішек микробиомасының сипаттамасын зерттеу.

Материалдар мен әдістері: бақылау, аналитикалық және Кросс-әдістер, ішек микробиотасының таксономиялық талдауы және NGS секвенирлеу әдісі.

Нәтижелер. Инсультпен және ИТ-мен ауыратын науқастардағы микробиотаның түрлік талдауы лактат түзетін Streptococcus бактерияларымен, бутират және ацетат түзетін бактериялармен Bacteroides (dorei, massiliensis, plebeius, tobetsuensis) және dialester invisus пропионат өндірушісі. Инсулинге төзімділігі бар емделушілерде инсульттің мұндай зерттелмеген болжамдарының болуы инсулинге төзімділігі бар емделушілерде қабынуды, қан қысымын және инсультті сақтауда ішек микробиотасының ықтимал әсері туралы айтады. Ацетат, бутират, пропионат өндірушісі бактериясының крахмалды белсенді ашыту кезіндегі рөлі көрсетілген, ол триглицеридтерге әсер етеді, содан кейін инсулинге төзімділік жоғарылайды. Қабыну және метаболизм жолдарын қамтитын хост-микробиотаның өзара әрекеттесуі жүрек-қан тамырлары ауруларының пайда болуында маңызды рөл атқарады. Патогенезде лактат, бутират және пропионат өндіретін бактериялар АГ-мен пайда болған кезде маңызды.

Қорытындылар. Нәтижелер инсультпен байланысты дисбиозбен ішек бактериялары шығаратын органикалық қышқылдардың тепе-теңдігі арасындағы ықтимал байланысты, атап айтқанда инсульттің ацетат пен бутират деңгейлерімен кері байланысын көрсетеді. Себептердің бірі дисбиозбен байланысты ішектің қабынуы болып табылады және жоғары қан қысымын сақтауға ықпал етеді.

Түйін сөздер: *микробиом, инсулинге төзімділік, артериялық гипертензия, инсульт.*

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Introduction

Dysbiosis, as changes in the composition and function of the intestinal microbiota (IM), is associated with stroke risk factors such as obesity, metabolic disorders, and atherosclerosis. In acute cerebral ischemia, IM plays a key role in the two-way interaction between the intestine and the brain, known as the microbiota-intestine-brain axis. The dysbiosis that exists before a stroke affects its outcomes, and during acute brain damage, the brain has a reverse effect on IM, which also affects the prognosis. These interactions are mediated by bacterial components (for example, lipopolysaccharides), IM metabolites (such as short-chain fatty acids and trimethylamine N-oxide), as well as through the immune and nervous systems. IM is affected by both genetic and environmental factors.

In general, IM is considered a stable system [6, 3], however, various factors, such as dietary and climatic changes, can influence it. Changes in the quantitative and qualitative composition of IM, leading to an increase in bacterial lipopolysaccharides, can also affect the expression of pro-inflammatory cytokines, causing the development of chronic sluggish inflammation [1, 8]. There is also evidence that food affects the composition of IM. For example, when switching from a diet enriched in fats and carbohydrates to a diet low in fat and rich in plant polysaccharides [25] and from a diet enriched in fats to a diet with a low glycemic index, noticeable changes in IM occur within 1 day [10, 26]. Currently, the problem of the cause-and-effect relationship between IM changes and obesity remains unresolved. Thus, understanding the mechanisms by which IM affects various signaling pathways and the phenotype of a microorganism can reveal new possible aspects of the prevention of obesity and type 2 diabetes [7]. The intestinal microbiota includes microorganisms living in the gastrointestinal tract. The intestinal microbiota of an adult consists of 10^{13}-10^{14} microorganisms/ml of the contents of the lumen, their total weight is estimated at 1.5 kg [19, 28]. The human gut microbiota consists of bacteria, archaea, fungi, protozoa, and viruses, among which bacteria dominate. The human microbiota genome includes 100 times more genes than the human genome and 10 times more cells than the human body [14]. The human microbiota is characterized by three groups of bacteria: symbionts, commensals, and pathobionts, which coexist in stable equilibrium in a healthy person. Symbionts have a healing effect, commensals demonstrate a neutral effect, i.e., they have neither a positive nor a negative effect, whereas pathobionts have the potential to cause pathology [13].

The human intestine is home to many microbes that form a complex microbial community, which is considered a vital organ. The intestinal microbiota forms a multidirectional connecting axis with other organs, interacting through nervous, endocrine, humoral, immunological, and metabolic pathways. Most intestinal microorganisms (mostly non-pathogenic) are in a symbiotic relationship with the human body, participating in immune defense against pathogens.

Dysbiosis of the intestinal microbiota is associated with a number of diseases, including anxiety and depressive disorders, hypertension, cardiovascular diseases, obesity, diabetes, inflammatory bowel diseases and cancer. The development of these diseases is associated with the microbiota, the products of its metabolism and the immune response of the organism. Although the mechanisms of the

positive and negative effects of microbiota on health remain poorly understood, recent clinical studies reveal a link between specific types of microorganisms, the state of eubiosis and the development of diseases. Understanding the interactions of the microbiota with the organism, its role in maintaining health and the pathogenesis of diseases, as well as relevant data on this topic are key aspects of the current analysis. Important tasks that need to be solved to maintain health and effective treatment are also highlighted. Intestinal dysbiosis is often observed in patients with acute ischemic stroke, which is associated with metabolism and inflammation in the host body, as well as with functional outcomes. Modulation of the intestinal microbiota or its metabolites improves conditions associated with the pathogenesis of stroke, including inflammation, cardiometabolic diseases, atherosclerosis, and thrombosis. Ischemic stroke is associated with an increase in the number of *Atopobium* and *Lactobacillus ruminis* cluster bacteria and a decrease in the number of *Lactobacillus sakei* subgroup [11, 20]. Arterial hypertension has an obvious induction effect on ischemic stroke, and the frequency and prognosis of ischemic stroke are closely related to the severity and duration of hypertension. Therefore, blood pressure is positively associated with the incidence of stroke [15]. There are studies confirming that arterial hypertension is one of the most independent risk factors for the development of ischemic stroke [5, 18]. The intestinal microbiota can produce substances that affect blood pressure levels that cannot be produced by other organs. Butyric acid and propionic acid can cause vasodilation of the colon and caudal arteries. Acetic acid is widely used in hemodialysis and is associated with the development of hypotension and vasodilation. A study by *Pleznick J.J.* showed that blood pressure in mice is regulated by SCFA (propionate) produced by the intestinal microbiota in response to antibiotic treatment. Thus, demonstrating how the abundance and diversity of the intestinal microbiota in rats with arterial hypertension and humans decreased significantly, and the proportion of Firmicutes/Bacteroidetes in rats with arterial hypertension increased [18]. Another study in mice with spontaneous hypertension demonstrated an increase in intestinal wall permeability, a decrease in the content of dense compound protein in the intestine, an increase in the number of intestinal *Streptococcus spp*. and a significant decrease in the number of *Bifidobacterium spp*. (SFCA, metabolites of the intestinal microbiota, can regulate blood pressure by activating receptors on the cell surface such as GPR41 and the olfactory receptor [17].

Aim of research: to study the characteristics of the intestinal microbiome in patients with stroke and insulin resistance.

Materials and Methods

The study is observational, analytical, and cross-sectional in nature with elements of taxonomic analysis of the intestinal microbiota and the NGS sequencing method for determining bacterial metagenomes based on 16sRNA data. The design of the study is "case-control".

The study was conducted on the basis of the the Laboratory for Personalized Genomic Diagnostics, Medical Center Hospital of the President's affairs Administration of the Republic of Kazakhstan during the implementation of the grant project "Study of the species structure of the intestinal microbiota in insulin resistance in the Kazakh population" (grant No. AR 14871581).

A cross-sectional case/control study was conducted with the participation of individuals from the Kazakh population assigned to the polyclinic in Astana. The study participants were selected by random sampling from people who visited the clinic for preventive purposes, the coverage period is January 2021 – March 2023.

Inclusion criteria: age 30-59 years, Kazakh nationality, voluntary consent to participate in the study. Exclusion criteria: persons of a different ethnic group, a history of chronic cardiometabolic and autoimmune diseases, antibiotic treatment during the last 3 months, pregnancy. All participants in the study signed an informed consent to participate in the study. The study was conducted in accordance with the principles of the Helsinki Declaration of the World Medical Association (1964). The project was approved by the Local Ethics Commission of the Medical Center Hospital of the President's affairs Administration of the RK, Protocol No.9 dated 09/19/2021.

The study participants were divided between the main (IR+) and control groups (IR-). Insulin resistance was determined using the triglyceride-glucose index (TyG), in which TyG = Ln [fasting glucose (mg/dl) × fasting triglycerides (mg/dl)]/2. The TyG index between 4.49 and 4.59 was defined as "moderate IR", the TyG index > 4.59 was assessed as "severe IR". The main group (IR+) included individuals with a TyG index of 4.5 and more. The number of participants in the main group was 199 people. The control group included individuals with a TyG index of 4.49 and less. There were only 17 participants in the control group. Thus, a group of 216 participants was formed for the study.

The material for the study was the fecal samples of the participants collected for microbiota analysis, as well as data from clinical and biochemical diagnostics. The samples were collected by the research participants. Each person was provided with a stool sampling kit, cotton swabs, and sterile paper towels, and the samples were frozen.

The species composition of the fecal microbiota of the research participants was studied by targeted semiconductor sequencing of the 16S rRNA gene of microorganisms using next-generation sequencing technology (NGS) using IonReporter software. A combination of two pools of primers was used, which made it possible to identify a wider range of bacteria based on the base sequence. Total DNA was extracted from a 250.0 mg homogenized wet fecal sample using the PureLink™ Microbiome DNA Purification Kit (TermoFisher Scientific, USA) in accordance with the manufacturer's instructions. The 16S rRNA gene was sequenced using a new generation semiconductor sequencer Ion Gene Studio S5 Plus (TermoFisher Scientific, USA) in the laboratory for personalized genomic diagnostics, Medical Center Hospital of the President's affairs Administration of the RK. DNA libraries (a set of nucleotide sequences of the genomic DNA of the studied samples) were prepared in accordance with the Ion 16S™ Metagenomics Kit protocol (Termofisher Scientific, USA). The library was prepared in several stages:

1. Obtaining a PCR product by amplification of the hypervariable 16S region, followed by purification and measurement of the concentration of the PCR product

2. Directly preparing the library by ligation with barcoding, purification of the adapter-ligated library

3. The sequence of amplified fragments was performed in the Ion PGM™ system, bioinformatic analysis of the results was carried out using the Ion Reporter ™ software, the Ion 16S ™ Metagenomics Kit analysis module.

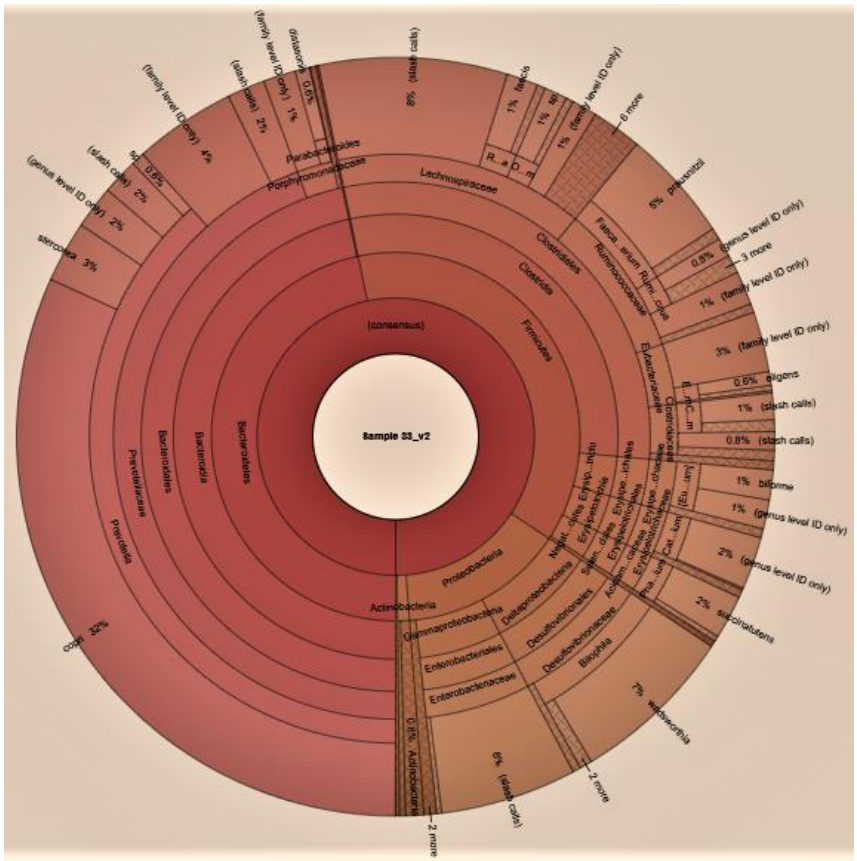
4. Using a combination of two pools of primers makes it possible to identify a wide range of bacteria in a mixed population by base sequence.

Further measurement of the concentration of the obtained library was determined on the "QuantStudio™ 12K Flex" system using the Ion Library TaqMan® Quantitation Kit (Termofisher Scientific, USA). Figure 1 shows an example of measuring library concentration.

Sequencing of the amplified fragments was performed in the Ion PGM ™ system, bioinformatic analysis of the results was carried out using the Ion Reporter ™ software, the Ion 16S ™ Metagenomics Kit analysis module.

Phylum	Class	Order	Family	Genus	Species	% ID	Count	DB counters	F:R %	% of total reads	% of valid reads	% of mapped reads	% of mapped reads per primer
							32474	**8761 : 23713**		**22.32**	**43.27**	**100**	**100**
Actinobacteria							335	216 : 119		0.23	0.45	1.03	1.03
	Actinobacteria						251	216 : 35		0.17	0.33	0.77	0.77
		Bifidobacteriales					97	81 : 16		0.07	0.13	0.3	0.3
			Bifidobacteriaceae				97	81 : 16		0.07	0.13	0.3	0.3
				Bifidobacterium			97	81 : 16		0.07	0.13	0.3	0.3
					(genus level ID only)		16	0 : 16		0.01	0.02	0.05	0.05
					(slash calls)		12	12 : 0		0.01	0.02	0.04	0.04
					catenulatum	99.55 - 99.55	50	50 : 0	0 : 100	0.03	0.07	0.15	0.15
					ruminantium	100 - 100	19	19 : 0	0 : 100	0.01	0.03	0.06	0.06
		Coriobacteriales					154	135 : 19		0.11	0.21	0.47	0.47
			Coriobacteriaceae				154	135 : 19		0.11	0.21	0.47	0.47
				(family level ID only)			19	0 : 19		0.01	0.03	0.06	0.06
				(slash calls)			33	33 : 0		0.02	0.04	0.1	0.1
				Collinsella			102	102 : 0		0.07	0.14	0.31	0.31
					aerofaciens	99.35 - 100	102	102 : 0	100 : 0	0.07	0.14	0.31	0.31

Picture 1. An example of bioinformatic analysis in tabular form



Picture 2. An example of bioinformatic analysis (Krohn diagram).

Figure 2 shows examples of bioinformatic analysis and a Crohn diagram.

Results

12 (6%) of the 199 patients with insulin resistance suffered a stroke, including 8 (66%) females and 4 (44%) males, with an average age of 52.2=7.0 years. 33 (16.6%) patients had type 2 diabetes mellitus, and 33 (16.6%) had coronary heart disease. Malignant neoplasms were noted in 115 (58%) patients, and 66 (33.3%) people were smokers. A taxonomic analysis was performed to identify predictors of stroke in patients with insulin resistance. The results are presented in table 1.

As can be seen from the materials in table 1, in stroke with insulin resistance, there is a decrease in bacterial diversity, predictors are represented in small numbers by a number of representatives of the *Bacteroides* family.

Таблица 1.

Stroke-associated bacteria in insulin resistance.

Type	Genus	p
Bacteroides	dorei	p≤0,05
	massiliensis	p≤0,0005
	plebeius	p≤0,01
Streptococcus		p≤0,05
Bacteroides	tobetsuensis	p≤0,0008
Dialester invisus		p≤0,02

A species analysis of the microbiota in patients with stroke and insulin resistance revealed a correlation with lactate-producing bacteria *Streptococcus* (p≤0,05), butyrate- and acetate-producing bacteria *Bacteroides (dorei, massiliensis, plebeius, tobetsuensis)* (p≤0,05) и *Dialester invisus* (p≤0,02), which is a producer of propionate. The results highlighted a possible link between stroke-related dysbiosis and the balance of organic acids produced by intestinal bacteria, namely, the inverse relationship of stroke with levels of acetate and butyrate.

Discussion

The presence of intestinal dysbiosis or a change in the composition of metabolites can lead to a more active synthesis of activity-dependent microorganisms, as well as to the induction of inflammatory reactions with systemic effects, which is known to underlie the pathogenesis of many chronic non-infectious diseases. One of the reasons is intestinal inflammation associated with dysbiosis and contributes to maintaining high blood pressure [12].

It is worth noting that the types of streptococcus found in the intestinal microbiota during stroke are associated with a number of pathologies based on inflammation [21]. In addition, *Streptococcus* bacteria contribute to the formation of lactate, which is directly related to the development of hypertension and stroke [12, 21]. In our study, a correlation was obtained (p≤0,05).

It is also impossible to exclude the effects of endotoxins. It has been proven that it is an excess of endotoxin that leads to a number of intercellular interactions and biochemical transformations, stimulating the development of systemic inflammatory response syndrome, endothelial dysfunction, dyslipidemia, hyperinsulinism, and atherogenesis, which are the basis for the progression of metabolic disorders, the development of systemic arterial hypertension and its dangerous complication stroke [22].

Ischemic stroke is associated with an increase in the number of *Atopobium* and *Lactobacillus ruminis* cluster bacteria and a decrease in the number of the *Lactobacillus sakei* subgroup [11]. Arterial hypertension has an obvious induction effect on ischemic stroke, and the frequency and prognosis of ischemic stroke are closely related to the severity and duration of hypertension. Therefore, blood pressure is positively associated with the incidence of stroke [15].

Of no small importance in the pathogenesis of stroke is insulin resistance [9], the degree of severity of which correlates with the accumulation of fats inside cells (liver, muscles, heart, vascular wall and other organs, concentrating in the peritoneum) in visceral obesity [24]. Evolutionarily, every human has the IR gene, which was originally an adaptation mechanism when periods of satiation alternated with periods of hunger. At the same time, the mechanisms of IR development are not completely known. The study of genetic factors has shown its polygenic nature.

Conclusions

In stroke complicated by insulin resistance, there is a decrease in intestinal microbial diversity and changes in the composition of the microbiota. A correlation was found with an increase in the number of lactate-producing bacteria (*Streptococcus*), as well as butyrate- and acetate-producing bacteria (*Bacteroides dorei, massiliensis, plebeius, tobetsuensis*) and the propionate-producing *Dialister invisus*.

The balance of organic acids (acetate, butyrate, propionate) produced by the intestinal microbiota is important in the pathogenesis of stroke. Disruption of this balance is associated with the activation of inflammatory reactions that support systemic inflammation and contribute to the development of chronic non-infectious diseases, including hypertension and stroke.

The excess of endotoxins produced by microorganisms contributes to the induction of a systemic inflammatory response, endothelial dysfunction, dyslipidemia, hyperinsulinism, and atherogenesis. These processes play a key role in the progression of metabolic disorders and the development of stroke.

An increase in the number of bacteria of the *Atopobium* and *Lactobacillus ruminis*, as well as a decrease in the *Lactobacillus sakei* subgroup, is associated with ischemic stroke. Arterial hypertension, in turn, has an inductive effect on stroke, and its frequency and prognosis depend on the severity and duration of hypertension.

Insulin resistance associated with visceral obesity and fat accumulation in organs is a significant risk factor for stroke.

Thus, the results highlighted a possible link between stroke-related dysbiosis and the balance of organic acids produced by intestinal bacteria, namely, the inverse relationship of stroke with the levels of acetate and butyrate. One of the reasons is intestinal inflammation associated with dysbiosis and contributes to maintaining high blood pressure.

Conflict of interests. *Not declared*

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Literature:

1. *Arkan M.C., Hevener A.L., Greten F.R. et al.* IKK-β links inflammation to obesity-induced insulin resistance. Nat. Med. 2005. № 11. PP. 191-198.

2. *Benakis C., Poon C., Lane D., et al.* Distinct commensal bacterial signature in the gut is associated with acute and long-term protection from ischemic stroke. Stroke. 2020, 51. pp. 1844–1854.

3. *Caporaso J.G., Lauber C.L., Costello E.K. et al.* Moving pictures of the human microbiome. Genom Biol. 2011.V.12. P. 50.

4. *Chen Y., Liang J., Ouyang F., et al.* Persistence of gut microbiota dysbiosis and chronic systemic inflammation after cerebral infarction in cynomolgus monkeys. Front. Neurol. 2019.V.10. pp.661.

5. *Cipolla M.J., Liebeskind D.S., Chan S.L.* The Importance of Comorbidities in Ischemic Stroke: Impact of Hypertension on the Cerebral Circulation. J. Cereb Blood Flow Metab. 2018. V.38(12). pp. 2129–49.

6. *Claesson M.J., Cusack S., O'Sullivan O. et al.* Composition, variability, and temporal stability of the intestinal microbiota of the elderly. Proc. Natl. Acad. Sci. USA. 2011, V. 108. pp. 4586-4591.

7. *Costello E.K., Lauber C.L., Hamady M. et al.* Bacterial community variation in human body habitats across space and time. Science. 2009. V. 326. pp. 1694-1697.

8. *Creely S., McTernan P.G., Kusminski C.M. et al.* Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. Am J Phys Endocrinol Metabol. 2007. V. 292. pp. E740- E747.

9. *Glass C.K., Olefsky J.M.* Inflammation and lipid signaling in the etiology of insulin resistance. Cell Metabol. 2012. № 15, V. 5. pp. 635-645.

10. *Jian S., Martin S.O., Liping Z.* The gut microbiota, obesity and insulin resistance. Mol Aspects Med. 2013. 34(1). pp.39-58. DOI: 10.1016/j.mam.2012.11.001.

11. *Jinchen W., Hongfei Zh., Jianying H. et al.* The Role of the Gut Microbiota in the Development of Ischemic Stroke. Immunol. 2022. 13 DOI:10.3389/fimmu.2022.845243.

12. *Kaindi D.W.M., Kogi-Makau W., Lule G.N. et al.* Colorectal cancer-associated Streptococcus infantarius subsp. infantarius differ from a major dairy lineage providing evidence for pathogenic, pathobiont and food-grade lineages. Sci. Rep. 2018. №8(1). p. 9181.

13. *Katsimichas T., Antonopoulos A.S., Katsimichas A. et al.* The intestinal microbiota and cardiovascular disease. Cardiovascular Research. 2019. DOI: 10.1093/cvr/cvz135.

14. *Koboziev I., Webb C.R., Furr K.L., Grisham M.B.* Role of the enteric microbiota in intestinal homeostasis and inflammation. Free Rad. Biol. Med. 2014. V.68. pp. 122-133.

15. *Li J., Zhao F., Wang Y., Chen J., Tao J., Tian G., et al.* Gut Microbiota Dysbiosis Contributes to the Development of Hypertension. Microbiome. 2017. V. 5(1). pp.14. DOI: 10.1186/s40168-016-0222-x.

16. *Ma J., Li H.* The role of gut microbiota in atherosclerosis and hypertension. Front Pharmacol. 2018. №9. p.1082. DOI:10.3389/fphar.2018.01082.

17. *Pluta R., Januszewski S.; Czuczwar S.J.* The Role of Gut Microbiota in an Ischemic Stroke. Int.J.Mol.Sci. 2021. V.22. p. 915. DOI: 10.3390/ijms22020915.

18. *Pluznick J.J.* Gut Microbes: A Novel SCFA Receptor, the Microbiota, and Blood Pressure Regulation. Gut Microbe. 2014. V.5(2). pp.202–207. DOI:10.4161/gmic.27492.

19. *Pokrzywnicka P., Gumprecht J.* Intestinal microbiota and its relationship with diabetes and obesity. Clin. Diabetol. 2016, V.*5*. pp. 164–172.

20. *Powers W.J., Clarke W.R., Grubb R.L., Videen T.O., Adams H.P., Derdeyn C.P.* Lower Stroke Risk With Lower Blood Pressure in Hemodynamic Cerebral Ischemia. Neurology. 2014. 82(12). pp.1027–1032. DOI: 10.1212/WNL.0000000000000238.

21. *Razavi A.C., Potts K.S., Kelly T.N., Bazanno L.A.* Sex, gut microbiome and cardiovascular disease risk // Biol. Sex. Differ. 2019. №10(1). p.29.

22. *Robles-Vera I., Toral M., de la Visitación N. et al.* Probiotics Prevent Dysbiosis and the Rise in Blood Pressure in Genetic Hypertension: Role of Short-Chain Fatty Acids. Mol. Nutr. Food Res. 2020. №64(6):e1900616.

23. *Singh V., Roth S., Lovera G., Sadler R., Garzetti D., Stecher B., Dichgans M., Liesz A.* Microbiota dysbiosis controls the neuroin flammatory response after stroke. J. Neurosci. 2016. V.36. PP.7428–7440. DOI:10.1523/JNeurosci.1114-16.2016.

24. *Snel M., Jonker J.T., Schoones J. et al.* Ectopic Fat and Insulin Resistance: Pathophysiology and Effect of Diet and Lifestyle Interventions. Intern J Endocrinol. 2012. pp.1-18.

25. *Turnbaugh P.J., Ridaura V.K. et al.* The effect of diet on the human gut microbiome: A metagenomic analysis in humanized gnotobiotic mice. Sci Trans Med. 2009. № 1. pp. 6-14.

26. *Wu G.D., Chen J., Hoffmann C. et al.* Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011. V. 334. pp. 105-108.

27. *Yin J., Liao S.X., He Y., Wang S., Xia G.H., Liu F.T., Zhu J.J., You C., Chen Q., Zhou L.* Dysbiosis of gut microbiota with reduced trimethylamine-n-oxide level in patients with large-artery atherosclerotic stroke or transient ischemic attack. J. Am. Heart Assoc. 2015. №4. e002699. DOI: 10.1161/JAHA.115.002699.

28. *Zhu S., Xu G.* Targeting gut microbiota: A potential promising therapy for diabetic kidney disease. Am. J. Transl. Res. 2016, V.8. pp. 4009–4016.

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