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LEVEL OF MIDDLE MOLECULAR PEPTIDES IN THE ARTICULAR CARTILAGE OF RATS UNDER EXPERIMENTAL OSTEOARTHRITIS AND ADMINISTRATION OF PROBIOTIC COMPOSITION

The question regarding clarification the mechanisms that link changes in the musculoskeletal system with changes in the microbiome, in recent few years has become particularly relevant. The investigation of the biological effect of probiotics on cartilage metabolism under osteoarthritis (OA) opens the perspectives for their use in complex therapy and prevention of joint pathology. The aim of the research was to analyze the quantitative and qualitative composition of the peptide component of the middle-mass molecules (MMM) of different fractions in the articular cartilage of rats with experimental OA under the introduction of multiprobiotic (PB) composition. The experiments were conducted on white male non-linear rats weighing 180-200 g. Experimental osteoarthritis in rats was induced by a single injection of sodium monoiodacetate (MIA; Sigma, USA) in the knee patellar ligament. The animals of therapeutic group received oral administration of live probiotic composition Multiprobiotic Simbiter® acidophilic concentrated ("O.D. Prolisok", Ukraine) at a dose of 140 mg/kg daily for 14 days. Euthanasia of animals was performed on the 30th day of the experiment. The level of MMM was assessed spectrophotometrically. Fractionation at the peptide level was performed using the method of chromatography, which is separated by size on Sephadex G 15 column. Our findings showed an increase in the MMM content of all studied fractions in cartilage tissue of rats with experimental OA and changes in the qualitative and quantitative composition of their the peptide component, that could indicate the development of endogenous intoxication, as a result of impaired cartilage metabolism, inflammation and destructive processes in the knee joint during the pathology. Administration to animals with MIA-induced OA PB composition had a favorable effect on the studied parameters, which was expressed in a decrease of MMM content and restored redistribution of the peptide pool of cartilage tissue. The obtained results provide grounds for further research aimed at studying the biological effect of PB on cartilage metabolism, which may contribute to the development of new strategies for the treatment and prevention of joint diseases.

Keywords: Osteoarthritis, middle-mass molecules, endogenous intoxication, peptide component, probiotic composition.

Introduction. Osteoarthritis (OA) is the most common disease among musculoskeletal pathologies, mainly among middle-aged and elderly people, and occupies a leading place among the global causes of disability [1]. More than 20 % of the world's population suffers from OA, while clinical symptoms of this pathology are recorded in about 18 % of women and 9.6 % of men over the age of 60 years [2]. The development of joint disease quite often occurs in combination with other pathologies of the musculoskeletal and cardiovascular system, diabetes mellitus, hypothalamic syndrome, pulmonary pathology, metabolic syndrome, obesity, etc., which can affect the course of OA [3-5]. Recently, the OA is considered as a whole organ disease in which all components of the joint are involved in the pathological process: cartilage, subchondral bone, synovial membrane, ligaments, capsules and periarticular muscles. The progression of OA is directly related to metabolic disorders, the formation of proteoglycan deficiency and uncontrolled inflammatory processes, resulting in chronic pain syndrome and endogenous intoxication [6, 7].

Endogenous intoxication (EI), which is associated with the accumulation in tissues and biological fluids of the organism of excess metabolites of normal and pathological metabolism, waste products of different pathogenic microorganisms is observed in various, etiologically and pathogenetically dissimilar conditions [8]. Under conditions of chronic endogenous intoxication, articular cartilage becomes a target for endogenous toxic compounds and the systemic effects mediated by them, in particular, mediators of the cytokine cascade, which results in disruption of the process of physiological regeneration and remodeling of cartilage tissue.

One of the most sensitive signs of EI is an increase in the level of so-called middle molecules (MMs, middle-mass molecules (MMM), middle molecular weight molecules (MMWM)), which mostly represented by peptides with a molecular weight of 300-5000 Da. MMs is a heterogeneous group of substances, the accumulation of which in the organism occurs in violation of the functional ability of detoxification systems and increased catabolism of proteins, nucleoproteins, some humoral regulators, which contributes to the defeat of toxic metabolites of the relevant organs and strengthening the course of the pathological process. Most of the MMs pool is formed by peptides, glycopeptides, endorphins, amino sugars, polyamines, insulin, glucagon, adrenocorticotropic hormone, vasopressin, oxytocin, angiotensin, lipofuscin, atherogenic lipoproteins, nucleotides, products of fibrinogen, albumin, thrombin degradation, collagen fragments, and derivatives of glucuronic acid, lipids, phospholipids, etc. The peculiarity of MMs is their high biological activity and tissue specificity. According to the research [9], identified a group of middle molecular weight peptides – fragments of collagen, which accelerate damage to the extracellular matrix and upregulate of matrix metalloproteinases (MMPs), which contribute to the destruction of cartilage during OA or may be processes in normal metabolic feedback [10, 11]. Today is widely studied the issue of using changes in the composition of the peptide pool of the organism to predict the course of diseases, in particular the cardiovascular system, liver and kidneys, metabolic syndrome, cancer and others [12, 13]. However, the pathogenetic role of MMs under the OA has not been sufficiently studied.

Meanwhile, the intensity and severity of endogenous intoxication depends not only on pathological processes that are accompanied by resistance of the organism, but also on the state of microbiocenoses. Since dysbiosis of the digestive system is observed in the development of many diseases of the organism, including pathologies of the musculoskeletal system [14, 15], so the question of maintaining a full-fledged microbiome today is given more and more attention. The authors of modern scientific research indicate that the state of the intestinal microbiome is an important pathogenic factor in the violation of bone homeostasis, and predict its role in the progression of OA [16, 17]. To restore and maintain the normobiosis of the gastrointestinal tract, are used the probiotics – drugs, based on living microorganisms and substances of microbial origin, which are producers of physiologically active metabolites

(vitamins, short-chain fatty acids, antioxidants and immunomodulators), which can reduce the development of inflammatory processes, detect antioxidant and detoxifying properties and cause positive effects on all systems of the organism [18]. Today it is extremely important to find and evaluate the effectiveness of new methods and means of prevention and treatment of OA, which will have a complex effect on articular tissues, have anti-inflammatory properties, especially in the early stages of the disease, reduce subchondral bone rigidity, improve joint lubrication, prevent the progression of cartilage degeneration and will contribute to the restoration (improvement) of joint function with minimal side effects.

Therefore, the aim of the study was to determine the level of middle molecules of different fractions and investigate the quantitative and qualitative composition of theire peptide component in the articular cartilage of rats with experimental osteoarthritis under the administration of multiprobiotic (PB) composition.

Materials and methods. The experiments were conducted on white male non-linear rats weighing 180-200 g, which were bred in the vivarium of Taras Shevchenko National University of Kyiv. The researches were performed according to the general rules and international bioethical principles of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, France, 1986), the General ethical principles, approved by the First National Congress of Ukraine on Bioethics (September, 2001), and other international agreements and national legislation in this field.

The all experimental animals were divided into three groups of 10 animals each. The rats of the Control group got injection of saline on the first day of experiment and oral administration of 1 ml drinking water daily from the 8th to the 21st day. In the animals of the second group (MIA-OA) modeled experimental osteoarthritis, which induced by single injection of 0.05 ml of sterile saline solution containing 1 mg sodium monoiodacetate (MIA; Sigma, USA) in the patellar ligament of left hind knee at the 1st day of the experiment [19]. The animals of third therapeutic group (MIA-OA+PB) got injection of MIA on the 1st day and received oral administration of live probiotic composition Simbiter® acidophilic concentrated (O.D. Prolisok, Ukraine) at a dose of 140 mg/kg dissolved in the 1 ml drinking water per 1 kg of the animal weight daily from the 8th to the 21st days of the experiment. Probiotic complex included live symbiotic biomass that contains 17 strains of microorganisms, belonging to the 10 genera: *Bifidobacterium*, *Lactobacillus*, *Propionibacterium*, *Lactococcus*, *Streptococcus* and *Acetobacter*. Multiprobiotic contains at least 1012 live cells of probiotic bacteria in one dose (10 ml).

Euthanasia of animals was performed for 30 day after the start of the experiment by decapitation according to the protocol of the ethics committee, after which the cartilage of the knee joints was taken in the cold for further research.

The level of middle-mass molecules was assessed spectrophotometrically according to the method of Nikolaychuk [20]. All manipulations were performed at a temperature of 4°C. To precipitate the bulk of proteins was added to the samples an equal volume of 1.2 M HClO4, followed by centrifugation for 20 min at 5000 g. The supernatant was neutralized by 5 N KOH to pH 7.0 and the samples were centrifuged again under the same conditions. After 60 % ethanol was added to the obtained supernatant in a ratio of 1:5, then the samples were incubated for 15 min and centrifuged at 2500 g. The optical density of the supernatants was measured with a spectrophotometer Smart SpecTMPlus (BioRad, USA) at a wavelength (λ) of 210 nm (the value of optical absorption is due to the presence of a peptide bond, MMM_1), 238 nm (absorption of aminopeptide fraction, MMM_2) and 254 nm (absorption of peptide fraction, which does not contain aromatic amino acids, MMM_3).

Fractionation at the peptide level was performed size exclusion chromatography via a column of Sephadex G 15 (Bio Rad, USA) [21]. The received samples of MMM were lyophilized (Testar Lyo Quest, Spain), then dissolved in 1 ml of 0.05M Tris-HCl buffer, pH 7.4, containing 0.13M NaCl. The chromatographic process rate was 18 ml/h. The column was pre-calibrated under similar conditions using standard markers solution such as lysozyme (14.3 kDa), insulin (5.7 kDa) and vitamin B12 (1.35 kDa).

Statistical analyses was performed using computer program OriginLab (v9.1). The research results were presented as average arithmetic ± standard deviation (dispersion) (M ± SD). The difference between of experimental groups was considered to be statistically significant at $p<0.05$.

Results and discussion. Articular cartilage is specialized connective tissue which unlike most tissues, does not have blood or lymphatic vessels and nerves. It is composed of a dense extracellular matrix (ECM), the main components of which are macromolecules of collagen (mainly type II) and proteoglycans (PG), including aggrecan, decorin, biglycan, and fibromodulin, with other noncollagenous proteins and glycoproteins present in lesser amounts which essential for normal function. Chondrocytes are the primary regulators of matrix anabolism and catabolism in articular cartilage. When this balance is disturbed and various biochemical pathways are activated, resulting in degradation of the extracellular matrix, inflammation occurs, and chondrocytes undergo dedifferentiation and hypertrophy, ultimately leading to OA [22]. Intoxication syndrome caused by the release of proteolytic enzymes stimulates increased tissue breakdown, increased catabolic processes due to the accumulation of excess amount of biologically active substances, deformed protein metabolites and other toxic substances of endogenous origin. In the tissues of the joint of patients with OA accumulate protein substances of different molecular weight, which are involved in the destruction of cartilage, tendons and ligaments [23, 24].

Our biochemical studies indicate the intensification of EI processes and pathological disturbance of articular cartilage homeostasis under the development of MIA-induced OA, as indicated by an increase in the level of MMM of different fractions in the cartilage tissue of the rat joint, compared to the Control (Fig. 1). Thus, in the group of animals in which was modeled of the experimental OA, the content of MMM_1, MMM_2 and MMM_3 increased by 3.9, 2.9 and 1.6 times respectively, compared to the values of control animals.

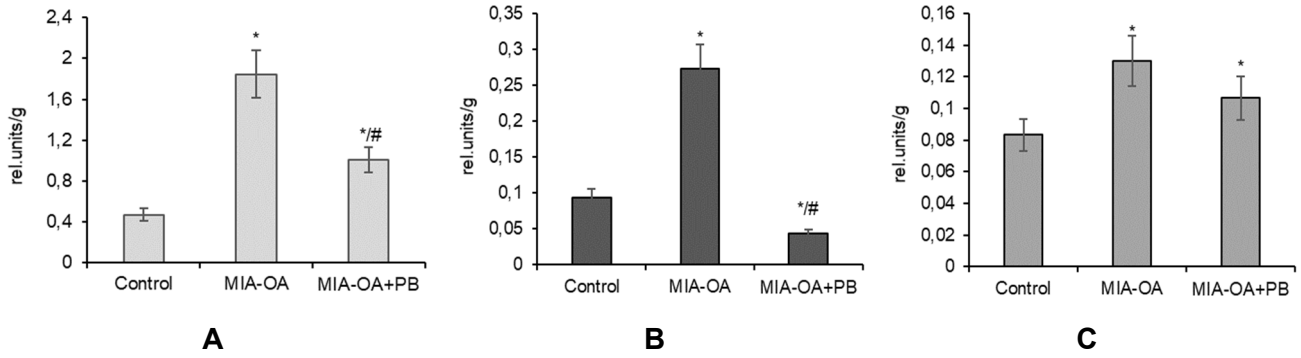

Fig. 1. The levels of middle-mass molecules MMM_1 (A), MMM_2 (B), MMM_3 (C) in articular cartilage of rats with experimental osteoarthritis (MIA-OA) under the administration of probiotic (PB) composition, relative (rel.) units/g (M±SD, n=10 in each group)

Notes: *p≤0.05 – statistically significant difference relative to Control group; #p≤0.05 – statistically significant difference relative to MIA-OA group.

It is worth noting that the breakdown of protein molecules, as a result of which MMM are formed, occurs under the action of proteinases. In studies [25] showed their prevalence, in particular at a wavelength of 254 nm, which testified to destructive-necrotic changes due to the elevation of the nucleoprotein component. Cartilage destruction is accompanied by increased levels of proteases such as adamalysin, disintegrin and MMP with thrombospondin type 1 (ADAMTSs). Among MMPs, the main factor in the destruction of collagen type 2 is MMP-13, other collagenases (MMP-1, MMP-8, MMP-2 and MMP-9) provide further cleavage of denatured collagen fibrils; MMP-3, ADAMTSs-4 and ADAMTSs-5 (aggrecanase) destroy the aggrecan [26]. The intensity of disintegration of biopolymers and the rate of their excretion through the detoxification organs, determines the level of MMM, respectively, the degree of EI, the duration and activity of the pathological process in the organism.

Endogenous intoxication and an increase in the content of MMM for OA is led by activation of free radical oxidation processes both at the local and systemic levels. As a result of the action of pro-inflammatory cytokines and activation of signaling cascades in cells are formed excess of reactive oxygen species (ROS), which results in increased lipid peroxidation (LPO), oxidative modification of proteins (OMP), destruction of nucleic acids, carbohydrates, ets [27]. Previous studies [28] showed that in the serum and cartilage tissue of rats with experimental joint pathology the content of superoxide radical and hydrogen peroxide, lipid peroxidation products and proteins increased, and the functioning of the antioxidant system was impaired.

Administration of the PB composition to rats with MIA-induced OA was accompanied by a decrease in the intensity of endotoxemia, as indicated by the following values of MMM content: the content of MMM_1 decreased by 1.8 times compared to animals of the MIA-OA group, but remained higher relative to Control by 2.1 times; the content of MMM_2 decreased by 6.3 times compared to animals of the MIA-OA group, and by 2.2 times – in relation to the control values; whereas the values of the content of MMM_3 did not differ significantly from the indicators of the MIA-OA group, although they tended to decrease (Fig. 1). Meddle-molecular peptide component, which are determined at a wavelength of 254 nm are toxic fraction and is an integral indicator of the content of ultraviolet absorbing substances, which, in addition to proteolysis products, include non-protein substances of normal and abnormal metabolism, change less significantly [29]. It is known that probiotics are able to reduce the permeability of tissue barriers to toxins, to provide detoxification of compounds formed in the organism as a result of pathogens. Multiprobiotics through the significant content of enzymes metabolize proteins, fats, carbohydrates, nucleic acids, as well as break down certain food and bacterial allergens [18]. Due to the wide range of biological activity, they promote to the production of mediators which have a positive effect not only on the functions of the gastrointestinal tract, but also on metabolic processes in the organism. The introduction of PB in animals with experimental joints pathology restored the disturbed oxidative-antioxidant balance both in the joint (locally) and at the systemic level (in the blood), reduced the intensity of inflammation in the organism, prevented the activation of proteolytic enzymes and the catabolism of collagen proteins, suppressed destructive processes in the tissues of the joint [28, 30].

The study of the qualitative composition of the protein component of the MMM cartilage of rats with experimental OA and the introduction of a PB composition was carried out using the chromatography method, dividing by size on a column with Sephadex G 15. The obtained results are showed in Fig. 2.

The presented chromatograms are typical for each study group, the difference is only in the number and parameters of chromatographic peaks. For quantitative characteristics of the obtained chromatograms, using markers of molecular weight, their calculations were performed. The data are presented in Table. 1.

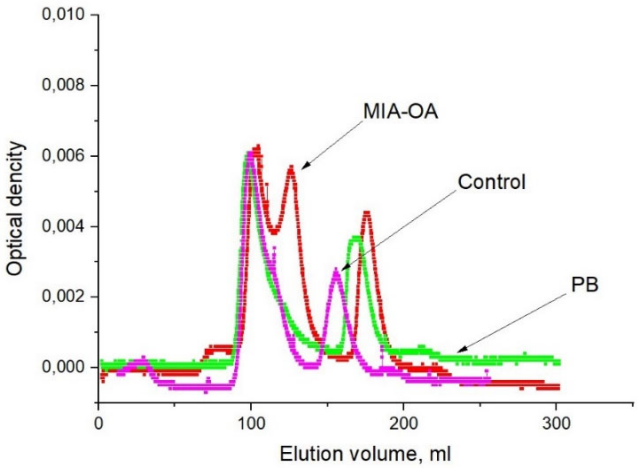

Fig. 2. The typical chromatograms showing the separation of the protein component of MMM articular cartilage of rats with MIA-induced osteoarthritis (MIA-OA) under administration of probiotic (PB) composition

***Table* 1. Characteristics of the qualitative composition of middle-mass molecules in homogenate of knee cartilage of rats with experimental osteoarthritis (MIA-OA) under administration of probiotic (PB) composition**

Investigated group	Peak number	Total area under peak (rel. units)	Molecular weight (Da)	Area under peak (rel. units)
Control	1 2 3	0,190	1602,0 955,8 1392,4	0,047 0,041 0,102
MIA-OA	1 2 3 4	0,264	1525,9 1269,4 893,4 1989,0	0,084 0,116 0,051 0,013
PB	1 2 3	0,161	1605,3 929,8 1422,1	0,052 0,046 0,063

The changes in the number of peaks and molecular weights of individual peptide fractions in the total pool of peptide component, which was applied to the chromatographic column, are shown. It was established an increase in the number of peaks and an increase in the variation of molecular masses in the cartilage tissue of rats with MIA-OA. According to the obtained results of chromatographic separation of the peptide component, in the articular cartilage of animals of the Control group was determined the presence of 3 fractions of peptides with a molecular weight of 1602.0, 1392.4 and 955.8 Da, the dominant quantitative ratio were peptides with a molecular weight of 1392.4 Da, the share of which in the total pool was about 54 %.

The peptide component obtained from the cartilage of rats with MIA-induced OA was divided into 4 fractions, the molecular weight of which was in range from 893.4 to 1989 Da. At the same time there was an increase in the total area of peaks in 1.4 times, compared to the animals of the control group, the dominance of peptides with a molecular weight of 1269.4 Da, the share of which in the total pool was about 44 %, the smallest share – about 5 %, were peptides with molecular weight 1989 Da.

In progressive diseases of the joint, the degradation of extracellular matrix proteins and proteoglycans leads to irreversible changes in the properties of the collagen network. In addition, the disbalance in the metabolism of matrix proteins often leads to enhanced proteolysis of molecules bound to and exposed on the surface of collagen fibers, such as fibromodulin, decorin and cartilaginous oligomeric matrix protein (COMP) [31].

The molecular weights of peptides accumulated in the cartilage tissue of animals with experimental OA, which received the PB composition were generally in the range of masses close to the values of the animals in the Control group and amounted to 1605.3, 1422.1 and 929.8 Da, with almost the same quantitative ratio in general pool of peptide component. Such obtained indicators may be related to the fact that the PB has anti-inflammatory, antioxidant and regenerative effects by both normalization of the intestinal microbiota and activation of anabolic processes in cartilage tissue under the conditions of osteoarthritis [32]. Consequently, showed a violation of the functioning of the proteolysis system, intensification of the degradation of protein molecules and their fragments, intensification of EI processes in the tissues of the rat joint under MIA-induced OA. The administration of PB composition to animals with experimental pathology had a favorable effect, which was manifested in the restored redistribution of the peptide pool of articular cartilage tissue. Determination of specific peptides in the tissues of the organism, including the musculoskeletal system, as well as qualitative and quantitative characteristics of individual peptide fractions, can be used as an indicator of the development of a pathological state, serve as a predicting marker of the course of the disease or the effectiveness of its therapy.

Conclusions. In the course of the conducted researches was analyzed the quantitative and qualitative composition of the protein component of the middle-mass molecules of different fractions in the cartilage tissue of rats with experimental OA under administration of a probiotic composition. The content of MMM_1, MMM_2 and MMM_3 was reliably increased in the cartilage tissue of rats with MIA-

induced OA, which could indicate endogenous intoxication, which may be result of impaired cartilage metabolism, inflammation and destructive processes in the knee joint during the development of pathology. Shown changes in the number of peaks and molecular weights of peptides in animal cartilage with experimental OA. The administration to animals with MIA-OA PB composition had a beneficial effect on the studied parameters, which was expressed in a decrease the content of MMM and restored redistribution of the peptide pool in cartilage tissue, which may be associated with a wide range of biological activity of this drug. Established changes in the qualitative and quantitative composition of peptide component of cartilage tissue of rats with experimental OA upon the aministration of probiotic composition can become the basis for further experiments aimed at identifying individual molecules as biomarkers of development and course of pathological process in the joints and expand scientific data on the biological action of probiotic on the organism under OA.

References

1. Hunter D.J. Osteoarthritis in 2020 and beyond: a Lancet Commission / D.J. Hunter, L. March, M. Chew // Lancet. – 2020. – Vol. 396(10264). – P.1711-1712.
2. Fekete H. Evaluation of osteoarthritis knee and hip quality of life (OAKHQoL): adaptation and validation of the questionnaire in the Hungarian population / H. Fekete, F. Guillemin, E. Pallagi [et al.] // Therapeutic Advances in Musculoskeletal Disease. – 2020. – Vol. 12. – P.1759720X20959570. doi: 10.1177/1759720X20959570.
3. Hall A.J. Association between osteoarthritis and cardiovascular disease: systematic review and meta-analysis / A.J. Hall, B. Stubbs, M.A. Mamas, P.K. Myint, T.O. Smith // European Journal of Preventive Cardiology. – 2016. – Vol. 23, №9. – P.938-946.
4. Zheng L. The role of metabolism in chondrocyte dysfunction and the progression of osteoarthritis / L. Zheng, Z. Zhang, P. Sheng, A. Mobasheri // Ageing Research Reviews. – 2021. – Vol. 66. – P.101249. doi: 10.1016/j.arr.2020.101249.
5. Tchetina E.V. insulin resistance in osteoarthritis: similar mechanisms to type 2 diabetes mellitus / E.V. Tchetina, G.A. Markova, E.P. Sharapova // Journal of Nutrition and Metabolism. – 2020. – Vol. 2020. – P.4143802. doi: 10.1155/2020/4143802.
6. Aspden M. Osteoarthritis as an organ disease: from the cradle to the grave / M. Aspden, F.R. Saunders // European Cells & Materials Journal. – 2019. – Vol. 37. – P.74-87.
7. Findlay D.M. Bone-cartilage crosstalk: a conversation for understanding osteoarthritis / D.M. Findlay, J.S. Kuliwaba // Bone Research. – 2016. – Vol. 4. – P.16028.
8. Sadikov R.A. Modern views and approaches to pathophysiological bases of endogenous intoxication / R.A. Sadikov, D.M. Khakimov, N.A. Kasimov // The Central Asian Journal of Medical Sciences. – 2021. – Vol. 2, №2. – P.140-148.
9. Schadow S. Collagen metabolism of human osteoarthritic articular cartilage as modulated by bovine collagen hydrolysates / S. Schadow, H.-C. Siebert, G. Lochnit, J. Kordelle, M. Rickert, J. Steinmeyer // PLoS One. – 2013. – Vol.8, №1. – P.e53955. https://doi.org/10.1371/journal.pone.0053955.
10. Schadow S. Metabolic response of human osteoarthritic cartilage to biochemically characterized collagen hydrolysates / S. Schadow, V.S. Simons, G. Lochnit [et al.] // International Journal of Molecular Sciences. – 2017. – Vol. 18, №1. – P.207. doi: 10.3390/ijms18010207.
11. Kumavat R. Biomarkers of joint damage in osteoarthritis: current status and future directions / R. Kumavat, V. Kumar, R. Malhotra [et al.] // Mediators of Inflammation. – 2021. – Vol. 2021. – P.5574582. https://doi.org/10.1155/2021/5574582.
12. Lygirou V. Plasma proteomic analysis reveals altered protein abundances in cardiovascular disease / V. Lygirou, A. Latosinska, M. Makridakis [et al.] // Journal of Translational Medicine. – 2018. – Vol. 16. – P.104. doi:10.1186/s12967-018-1476-9.
13. Dersh D. A few good peptides: MHC class I-based cancer immunosurveillance and immunoevasion / D. Dersh, J. Hollý, J.W. Yewdell // Nature Reviews Immunology. – 2021. – Vol. 21. – P.116-128.
14. Li R. The gut microbiome: a new frontier in musculoskeletal resear / R. Li, C.G. Boer, L. Oei [et al.] // Current Osteoporosis Reports. – 2021. – P.19:347-357.
15. Gizard F. Interactions between gut microbiota and skeletal muscle / F. Gizard, A. Fernandez, F. De Vadder // Nutrition and Metabolic Insights. – 2020. – Vol. 13. – P.1178638820980490. doi: 10.1177/1178638820980490.
16. Favazzoa L.J. The gut microbiome-joint connection: implications in osteoarthritis / L.J. Favazzoa, H. Hendesia, D.A. Villania // Current Opinion in Rheumatology. – 2020. – Vol. 32, №1. – P.92-101.
17. Sánchez Romero E.A. Relationship between the gut microbiome and osteoarthritis pain: review of the literature / E.A. Sánchez Romero, E.M. Oliva, J.L.A. Pérez [et al.] // Nutrients. – 2021. – Vol. 3, №3. – P.716.
18. Xu H. The dynamic interplay between the gut microbiota and autoimmune diseases / H. Xu, M. Liu, J. Cao [et al.] // Journal of Immunology Research. – 2019. – Vol. 27. – P.7546047.
19. Baragi V.M. A new class of potent matrix metalloproteinase 13 inhibitors for potential treatment of osteoarthritis: evidence of histologic and clinical efficacy without musculoskeletal toxicity in rat models / V.M. Baragi, G. Becher, A.M. Bendele [et al.] // Arthritis & Rheumatology. – 2009. – Vol. 60, №7. – P.2008-2018.
20. Nykolaychyk B.B. Method for determining of the peptide pool molecular / B.B. Nykolaychyk, V.M. Moyn, V.V. Kyrkovskyy // Laboratory case. – 1991. – Vol. 10. – P.13-18. [in Russian].
21. Paula H. Size-exclusion chromatography for the analysis of protein biotherapeutics and their aggregates / H. Paula, K. Stephan, E. Edouard // Journal of Liquid Chromatography & Related Technologies – 2012. – Vol.35. – P.2923-2950.
22. Meo Burt P. FGF23 Regulates Wnt/β-catenin signaling-mediated osteoarthritis in mice over-expressing high-molecular-weight FGF2 / P. Meo Burt, L. Xiao, M.M. Hurley // Endocrinology. – 2018. – Vol. 159, №6. – P.2386-2396.
23. Dickinson S.C. Cleavage of car-tilage oligomeric matrix protein (thrombospondin-5) by matrix metalloproteinases and a disintegrin and metalloproteinase with thrombospondin motifs / S.C. Dickinson, M.N. Vankemmelbeke, D.J. Buttle, K. Rosenberg, D. Heinegard, A.P. Hollander // Matrix Biology. – 2013. – Vol. 22, №3. – P.267-278.
24. Sheridan C. Low-molecular-weight albumin drug touted for severe osteoarthritis / C. Sheridan // Nature Biotechnology. – 2018. – Vol.36, №4. – P.293. doi: 10.1038/nbt0418-293.
25. Cherkasova V.V. Role of endogenous intox-ication in acute experimental pancreatitis / V.V. Cherkasova // Art of Medicine. – 2018. – Vol. 2, №1. – P.34-37.
26. Duan L. Recent progress on the role of miR-140 in cartilage matrix remodelling and its implications for osteoarthritis treatment / L. Duan, Y. Liang, X. Xu [et al.] // Arthritis Research and Therapy. – 2020. – Vol. 22. – P.194. https://doi.org/10.1186/s13075-020-02290-0.
27. Korotkyi O. Lipid peroxidation in rat cartilage under experimental osteoarthritis and administration of multiprobiotic / O. Korotkyi, L. Kot, K. Dvorshchenko. Visnyk Taras Shevchenko National University Of Kyiv. Biology. – 2020. – Vol. 80, №1. – P.41-44. [Ukrainian].
28. Korotkyi O. Oxidative/antioxidant balance and matrix metalloproteinases level in the knee cartilage of rats under experimental osteoarthritis and probiotic administration / O. Korotkyi, K. Dvorshchenko, L. Kot, T. Vovk, M. Tymoshenko, L. Ostapchenko // The Ukrainian Biochemical Journal. – 2020. – Vol. 92, №6. – P.26-136.
29. Kuzminova E. The spectrum of molecules of medium mass as endogenous intoxication indicator in quails with mycotoxicosis / E. Kuzminova, E. Dolgov, M. Semenenko, D. Osepchuk, K. Semenenko // E3S Web of Conferences. – 2020. – Vol. 210. – P.06010. https://doi.org/10.1051/e3sconf/202021006010.
30. Korotkyi O. Effect of probiotic composition on oxidative/antioxidant balance in blood of rats under experimental osteoarthritis / O. Korotkyi, K. Dvorshchenko, A. Vovk, A. Dranitsina, M. Tymoshenko, L. Kot, L. Ostapchenko // The Ukrainian Biochemical Journal. – 2019. – Vol. 91, №6. – P.49-58.
31. Åhrman E. Novel cartilage oligomeric matrix protein (COMP) neoepitopes identified in synovial fluids from patients with joint diseases using affinity chromatography an mass spectrometry. E. Åhrman, P. Loreno, K. Holmgren [et al.] // JBC Papers in Press. – 2014. doi 10.1074/jbc.M114.554683.
32. Cristofori F. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body / F. Cristofori, V.N. Dargenio, C. Dargenio, V.L. Miniello, M. Barone, R. Francavilla // Frontiers in Immunology. – 2021. https://doi.org/10.3389/fimmu.2021.578386.

References (Scopus)

1. Hunter DJ, March L, Chew M. Osteoarthritis in 2020 and beyond: a Lancet Commission. Lancet. 2020;396(10264):1711-1712. doi: 10.1016/S0140-6736(20)32230-3.
2. Fekete H, Guillemin F, Pallagi E, Fekete R, Lippai Z, et al. Evaluation of osteoarthritis knee and hip quality of life (OAKHQoL): adaptation and validation of the questionnaire in the Hungarian population. Ther Adv Musculoskelet Dis. 2020;12:1759720X20959570. doi: 10.1177/1759720X20959570.
3. Hall AJ, Stubbs B, Mamas MA, Myint PK, Smith TO. Association between osteoarthritis and cardiovascular disease: systematic review and meta-analysis. Eur J Prev Cardiol. 2016;23(9):938-946.
4. Zheng L, Zhang Z, Sheng P, Mobasheri A. The role of metabolism in chondrocyte dysfunction and the progression of osteoarthritis. Ageing Res Rev. 2021;66:101249. doi: 10.1016/j.arr.2020.101249.
5. Tchetina EV, Markova GA, Sharapova EP. Insulin resistance in osteoarthritis: similar mechanisms to type 2 diabetes mellitus. J nutr metab. 2020;2020:4143802. doi: 10.1155/2020/4143802.

6. Aspden M, Saunders FR. Osteoarthritis as an organ disease: from the cradle to the grave. Eur Cell Mater. 2019;37:74-87.

7. Findlay DM, Kuliwaba JS. Bone-cartilage crosstalk: a conversation for understanding osteoarthritis. Bone Res. 2016;4:16028.

8. Sadikov RA, Khakimov DM, Kasimov NA. Modern views and approaches to pathophysiological bases of endogenous intoxication. Central Asian J Med Nat Sci, 2021;2(2)P:140-148.

9. Schadow S, Siebert H-C, Lochnit G, Kordelle J, Rickert M, Steinmeyer J. Collagen metabolism of human osteoarthritic articular cartilage as modulated by bovine collagen hydrolysates. PLoS One. 2013;8(1):e53955. https://doi.org/10.1371/journal.pone.0053955.

10. Schadow S, Simons VS, Lochnit G, Kordelle J, GazovaZ, et al. Metabolic response of human osteoarthritic cartilage to biochemically characterized collagen hydrolysates. Int J Mol Sci. 2017;18(1):207. doi: 10.3390/ijms18010207.

11. Kumavat R, Kumar V, Malhotra R, Pandit H, Jones E, Ponchel F, Biswas S. Biomarkers of joint damage in osteoarthritis: current status and future directions. Mediators of Inflammation. 2021;2021:5574582. https://doi.org/10.1155/2021/5574582.

12. Lygirou V, Latosinska A, Makridakis M, et al. Plasma proteomic analysis reveals altered protein abundances in cardiovascular disease. J Transl Med. 2018;16:104. doi:10.1186/s12967-018-1476-9.

13. Dersh D, Hollý J, Yewdell J.W. A few good peptides: MHC class I-based cancer immunosurveillance and immunoevasion. Nat Rev Immunol. 2021;21:116-128.

14. Li R, Boer CG, Oei L, et al. The gut microbiome: a new frontier in musculoskeletal research. Curr Osteoporos Rep. 2021;19:347-357.

15. Gizard F, Fernandez A, De Vadder F. Interactions between gut microbiota and skeletal muscle. Nutr Metab Insights. 2020;13:1178638820 980490. doi: 10.1177/1178638820980490.

16. Favazzoa LJ, Hendesia H, Villania DA. The gut microbiome-joint connection: implications in osteoarthritis. Cur Opin Rheumatol. 2020;32(1):92-101.

17. Sánchez Romero EA, Oliva EM, Pérez JLA, Pérez SM, Turroni S, Marchese L, Villafañe JH. Relationship between the gut microbiome and osteoarthritis pain: review of the literature. Nutrients. 2021;13(3):716.

18. Xu H, Liu M, Cao J, Li X, Fan D, Xia Y, Lu X, Li J, Ju D, Zhao H. The dynamic interplay between the gut microbiota and autoimmune diseases. J Immunol Res. 2019;27;2019:7546047.

19. Baragi VM, Becher G, Bendele AM, Biesinger R, Bluhm H, et al. A new class of potent matrix metalloproteinase 13 inhibitors for potential treatment of osteoarthritis: evidence of histologic and clinical efficacy without musculoskeletal toxicity in rat models. Arthritis Rheum. 2009;60(7):2008-2018.

20. Nykolaychyk BB, Moyn VM, Kyrkovskyy VV. Method for determining of the peptide pool molecular. Laboratory case. 1991;10:13-18. [in Russian].

21. Paula H, Stephan K, Edouard E. Size-exclusion chromatography for the analysis of protein biotherapeutics and their aggregates. J Liquid Chromatography and Related Technologies. 2012;35:2923-2950.

22. Meo Burt P, Xiao L, Hurley MM. FGF23 Regulates Wnt/β-catenin signaling-mediated osteoarthritis in mice over-expressing high-molecular-weight FGF2. Endocrinology. 2018;159(6):2386-2396.

23. Dickinson SC, Vankemmelbeke MN, Buttle DJ, Rosenberg K, Heinegard D, Hollander AP. Cleavage of car-tilage oligomeric matrix protein (thrombospondin-5) by matrix metalloproteinases and a disintegrin and metalloproteinase with thrombospondin motifs. Matrix Biol. 2013;22(3):267-278.

24. Sheridan C. Low-molecular-weight albumin drug touted for severe osteoarthritis. Nat. Biotechnol. 2018;36(4):293. doi: 10.1038/nbt0418-293.

25. Cherkasova VV. Role of endogenous intox-ication in acute experimental pancreatitis. Art of Medicine. 2018;2(1):34-37.

26. Duan L, Liang Y, Xu X, et al. Recent progress on the role of miR-140 in cartilage matrix remodelling and its implications for osteoarthritis treatment. Arthritis Res Ther. 2020;22:194. https://doi.org/10.1186/s13075-020-02290-0.

27. Korotkyi O, Kot L, Dvorshchenko K. Lipid peroxidation in rat cartilage under experimental osteoarthritis and administration of multiprobiotic. Visnyk Taras Shevchenko National University Of Kyiv. Biology. 2020;80(1):41-44. [Ukrainian].

28. Korotkyi O, Dvorshchenko K, Kot L, Vovk T, Tymoshenko M, Ostapchenko L. Oxidative/antioxidant balance and matrix metalloproteinases level in the knee cartilage of rats under experimental osteoarthritis and probiotic administration. Ukr Biochem J. 2020;92(6):126-136.

29. Kuzminova E, Dolgov E, Semenenko M, Osepchuk D, Semenenko K. The spectrum of molecules of medium mass as endogenous intoxication indicator in quails with mycotoxicosis. E3S Web of Conferences 2020;210:06010. https://doi.org/10.1051/e3sconf/202021006010.

30. Korotkyi O, Dvorshchenko K, Vovk A, Dranitsina A, Tymoshenko M, Kot L, Ostapchenko L. Effect of probiotic composition on oxidative/antioxidant balance in blood of rats under experimental osteoarthritis. Ukr Biochem J. 2019;91(6):49-58.

31. Åhrman E, Lorenzo P, Holmgren K, Grodzinsky AJ, Dahlberg LE, Heinegård TSD, Önnerfjord P. Novel cartilage oligomeric matrix protein (COMP) neoepitopes identified in synovial fluids from patients with joint diseases using affinity chromatography an mass spectrometry. JBC Papers in Press, 2014. doi 10.1074/jbc.M114.554683.

32. Cristofori F, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. Front. Immunol, 2021. https://doi.org/10.3389/fimmu.2021.578386.

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РІВЕНЬ СЕРЕДНЬОМОЛЕКУЛЯРНИХ ПЕПТИДІВ У ХРЯЩОВІЙ ТКАНИНІ СУГЛОБА ЩУРІВ ЗА ЕКСПЕРИМЕНТАЛЬНОГО ОСТЕОАРТРИТУ ПРИ ВВЕДЕННІ ПРОБІОТИЧНОЇ КОМПОЗИЦІЇ

Питання щодо з'ясування механізмів, які пов'язують зміни в кістково-м'язовій системі зі змінами в мікробіомі, за останні кілька років набуває особливої актуальності. Вивчення біологічної дії пробіотиків на хрящовий метаболізм за розвитку остеоартриту (ОА) відкриває перспективи їх використання у комплексній терапії та профілактиці патології суглобів. Метою роботи було проаналізувати кількісний і якісний склад пептидної складової молекул середньої маси (МСМ) різних фракцій у хрящовій тканині колінного суглоба щурів з експериментальним ОА за введення мультипробіотичної (ПБ) композиції. Дослідження проводили на білих нелінійних щурах-самцях масою 180-200 г. Модель експериментального ОА у щурів створювали шляхом одноразового введення в інфрапателярний лігамент колінного суглоба монойодацетату натрію (МЙА; Sigma, США). Тварини терапевтичної групи щоденно протягом 14 днів перорально у дозі 140 мг/кг отримували живу пробіотичну композицію "Мультипробіотик Симбітер® ацидофільний концентрований" ("О. Д. Пролісок", Україна). Евтаназію тварин проводили на 30-ту добу експерименту. Рівень МСМ оцінювали спектрофотометрично. Фракціонування на пептидному рівні здійснювали методом хроматографії, що поділяє за розмірами, на колонці із Sephadex G 15. Показано збільшення вмісту МСМ усіх досліджуваних фракцій у хрящовій тканині щурів із експериментальним ОА та встановлено зміни якісного і кількісного складу їхньої пептидної складової, що могло свідчити про розвиток ендогенної інтоксикації унаслідок порушення метаболізму хряща, запалення та деструктивних процесів у колінному суглобі під час розвитку патології. Введення тваринам із МЙА-індукованим ОА мультипробіотичної композиції мало сприятливий ефект на досліджувані параметри, який виражався у зниженні вмісту МСМ та відновленні перерозподілу пептидного пулу хрящової тканини. Отримані результати дають підстави для проведення подальших досліджень, спрямованих на вивчення біологічної дії ПБ на метаболізм хряща, що може сприяти розробленню нових стратегій лікування та профілактики захворювань суглобів.

Ключові слова: остеоартрит, молекули середньої маси, ендогенна інтоксикація, пептидна складова, пробіотична композиція.

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УРОВЕНЬ СРЕДНЕМОЛЕКУЛЯРНЫХ ПЕПТИДОВ В ХРЯЩЕВОЙ ТКАНИ СУСТАВА КРЫС С ЭКСПЕРИМЕНТАЛЬНЫМ ОСТЕОАРТРИТОМ ПРИ ВВЕДЕНИИ ПРОБИОТИЧЕСКОЙ КОМПОЗИЦИИ

Вопрос по выяснению механизмов, связывающих изменения в костно-мышечной системе с изменениями в микробиоме, за последние несколько лет приобретает особую актуальность. Изучение биологического действия пробиотиков на хрящевой метаболизм при развитии остеоартрита (ОА) открывает перспективы их использования в комплексной терапии и профилактике патологии суставов. Целью работы было проанализировать количественный и качественный состав пептидной составляющей молекул средней массы (МСМ) разных фракций в хрящевой ткани коленного сустава крыс с экспериментальным ОА при введении мультипробиотической (ПБ) композиции. Исследования проводили на белых нелинейных крысах-самцах массой 180-200 г. Модель экспериментального ОА у крыс создавали путем однократного введения в инфрапателярный лигамент коленного сустава монойодацетата натрия (МЙА; Sigma, США). Животные терапевтической группы ежедневно в течение 14 дней перорально в дозе 140 мг/кг получали живую пробиотическую композицию "Мультипробиотик Симбитер® ацидофильный концентрированный" ("О. Д. Пролисок", Украина). Эвтаназию животных проводили на 30-е сутки эксперимента. Уровень МСМ оценивали спектрофотометрически. Фракционирование на пептидном уровне осуществляли методом хроматографии, разделяющей по размерам, на колонке из Sephadex G 15.

Показано увеличение содержания МСМ всех исследуемых фракций в хрящевой ткани крыс с экспериментальным ОА, а также установлены изменения качественного и количественного состава их пептидной составляющей, что могло свидетельствовать о развитии эндогенной интоксикации вследствие нарушения метаболизма хряща, воспаления и деструктивных процессов в коленном суставе при развитии патологии. Введение животным с МЙА-индуцированным ОА мультипробиотической композиции имело благоприятный эффект на исследуемые показатели, выражавшийся в снижении содержания МСМ и восстановлении перераспределения пептидного пула хрящевой ткани. Полученные результаты дают основания для проведения дальнейших исследований, направленных на изучение биологического действия ПБ на метаболизм хряща, что может способствовать разработке новых стратегий лечения и профилактики заболеваний суставов.

Ключевые слова: остеоартрит, молекулы средней массы, эндогенная интоксикация, пептидная составляющая, пробиотическая композиция.