Peptides in complex therapy of musculoskeletal pathology: clinical and molecular aspects

The search of new methods of therapy of the musculoskeletal system diseases (osteoarthritis, osteoarthrosis, osteoporosis, osteochondrosis) is an urgent task of gerontology and molecular medicine due to the high prevalence of these pathologies. It is particularly necessary to emphasize the medical and social significance of diseases of the musculoskeletal system, since they significantly reduce the quality of life (pronounced pain syndrome), mobility, efficiency and social activity of middle-aged and elderly people. According to the Ministry of Labor of Russia and Rosstat, diseases of the musculoskeletal system are the cause of an increase in the severity of other concomitant pathologies: cardiovascular, diabetes mellitus, chronic obstructive pulmonary disease, senile asthenia. Osteoarthritis has been the leading cause of disability among adults for the past 15 years.

Maintaining of the functional activity of the elements of the musculoskeletal system (chondroprotection) offers preventive strategies that can slow the progression of joint pathology and loss of their functions, as well as reduce the severity of pain syndrome by affecting the signaling pathways of inflammation. However, the success of such strategies requires an integrated approach that provides a targeted impact on the cause of the disease at the level of tissues and cells of the joint. The polypeptide complex of cartilage (Sigumir®) and a tripeptide AED (Cartalax®) were created at the St. Petersburg Institute of Bioregulation and Gerontology and can solve this problem.

Sigumir® is a polypeptide complex obtained by extraction from the cartilage and bone tissues of young animals. Sigumir® is a biologically active food supplement in the form of capsules. The composition of Sigumir® includes peptides with a molecular weight from 75 to 10,000 Da. Sigumir® is recommended for prevention and maintenance therapy of diseases of the musculoskeletal system: arthrosis and arthritis, rheumatism, osteochondrosis, osteoporosis, gout, etc.

The composition of the cartilage polypeptide complex was analyzed at the Institute of Toxicology of the FMBA of Russia using matrix-activated laser

desorption/ionization (MALDI) and ultra-efficient liquid chromatomass spectrometry (HPLC-MS). The tripeptide AED (Alanine-Glutamine-Asparagine) was found in the compound of the polypeptide complex of cartilage. The AED peptide has biological activity similar to Sigumir® [Zhurkovich I.K. et al., 2020]. The AED tripeptide is produced as a biologically active food additive Cartalax®.

Cartalax® is recommended for the prevention and complex therapy of the following diseases: arthrosis and arthritis, osteochondrosis, osteoporosis, degenerative-dystrophic joint diseases, rheumatism, the consequences of joint injuries, gout, systemic prevention of bone and joint injuries in sports, systemic connective tissue diseases, preoperative and postoperative periods during joint operations, prevention of degenerative processes in spine and joints in elderly and old people. Since the tripeptide is a small molecule, it is not recognized by the cells of the immune system and does not cause allergic reactions and other side effects. This circumstance is especially important in the treatment of patients of older age groups.

The reparative effect of the polypeptide complex extracted from cartilage and bone tissue in relation to bone tissue was revealed in two experimental models of traumatic fracture of old rabbits. In the experimental group, daily applications were made to the site of the bone defect with a polypeptide complex of cartilage and bone tissue at a dose of 0.7 mg /kg dissolved in 2 ml of saline solution for 5 days. Healing took place naturally in the animals of the control group. The formation of a full-fledged flat spongy bone was observed on the 28th day after the application of the polypeptide complex. The bone defect persisted in the control group at this time. In the second experiment, a Teflon fistula was injected into the thigh of rabbits under anesthesia, which was fixed to the femur. This design of the experiment allows us to evaluate the migration of bone marrow elements into the resulting void space. Animals of the experimental group from the 1st to the 7th day of the investigation were injected into the lumen of the fistula with a polypeptide complex of cartilage and bone tissue at a dose of 0.7 mg in 1 ml of saline solution. the formation of bone tissue was observed in the 3rd week of the experiment under the action of the

polypeptide complex. In the control group (administration of saline solution), this effect was achieved later, only by the end of the 4th week of the study [Ryzhak G.A. et al., 2019].

The development of posttraumatic osteoarthritis in rats was simulated in another experiment. Animals were injured in the area of the inner condyle of the femur. Rats had degenerative-dystrophic changes in the cartilaginous tissue of the articular surface, characteristic of osteoarthritis on the 5th day of the experiment. Animals of experimental groups received intramuscularly polypeptide complex of cartilage and bone tissue in doses of 0.02 mg or 0.2 mg in 0.4 ml of saline solution 1 time a day during 10 days. Rats in the control group were injected with 0.4 ml of saline solution according to the same scheme. The structure of cartilage tissue was restored under the action of a polypeptide complex of cartilage and bone tissues on the 28th day of the experiment [Ryzhak G.A. et al., 2019].

Another experimental study showed the restoration of bone mineral density in rats after ovariectomy (model of osteoporosis) under the action of a polypeptide complex of cartilage and bone tissue and the tripeptide AED. The study was conducted on 100 Wistar rats (mature females). Osteoporosis was modeled by ovariectomy (removal of the ovaries). The decrease of bone mineral density was observed in rats according to densitometry during one month after ovariectomy. Rats were divided into several groups: 1 - control (without surgery, saline injection), 2 – ovariectomy, 3 – Cartalax® (10 mcg), starting from the 4th day after ovariectomy, 4 – Cartalax® (10 mcg), starting from the 30th day after ovariectomy, 5 - Sigumir® (1 mg), starting from the 4th day after ovariectomy, 6 - Sigumir® (1 mg), starting from the 30th day after ovariectomy. Sigumir® applying, starting from the 30th day after ovariectomy, increased the reduced bone mineral density, and a month after the end of the drug administration, the achieved effect was maintained. Cartalax® has no this long effect, although the mineral density of the bone tissue increased during the use of this tripeptide [Povoroznyuk V.V. et al., 2007]. The results of this experimental study formed the basis for the recommendation of long-term use of

Cartalax® and Sigumir® for the prevention of osteoporosis, especially in women over 50 years old.

It was shown the positive effect of Cartalax® on calcium metabolism in bone tissue. Violation of calcium metabolism in bone tissue leads to the development of osteoporosis. The effect of Cartalax® on the morpho-functional organization of calcitonin-producing thyroid cells of epiphysectomized rats was studied in the experiment. Epiphysectomy is the removal of the epiphysis, the central organ of the neuro-immuno-endocrine system. This operation leads to a violation of the functions of the thyroid gland and the development of osteoporosis. Wistar rats were injected with Cartalax® during 21 days after epiphysectomy at a dose of 0.5 mcg per rat for 10 days (experimental group). Control animals were injected with saline solution according to the same scheme. restoration of the thyroid tissue structure was observed in rats after epiphysectomy on the 3^{rd}-12^{th} days after the end of Cartalax® injections. Cartalax® contributed to an increase of the number of thyroid C cells and the restoration of their function, which indicates an increase in the process of calcium resorption in bone tissue. These data may be important in the treatment of osteoporosis.

What is the molecular mechanism of action of the polypeptide complex of cartilage and bone tissues (Sigumir®) and the tripeptide Cartalax®? To answer this question, studies were conducted in cartilage tissue cultures of young and old rats. The growth of cartilage cells (chondrocytes) was assessed by the area index. This is the ratio of the sum of the area of the central zone of the explant (fragment of cartilage) and the peripheral zone (cells formed next to the original fragment of cartilage by division) to the area of the central zone in %. It was found that both peptide drugs increased the cartilage area index by 18-38%. In addition, Sigumir® and Cartalax® stimulated the synthesis of the molecular marker of proliferation (growth) of cartilage cells – PCNA and reduced the synthesis of the p53 protein, the trigger factor of programmed cell death (apoptosis) [Smirnov A.V. et al., 2011].

The activation of p53 gene induces apoptosis and aging of chondrocytes. Oxidative stress caused by an inflammatory reaction in osteoarthritis increases the

expression of p53. Suppression of p53 expression prevents apoptosis of chondrocytes. PCNA (nuclear antigen of a proliferating cell) is the protein that acts as a cofactor of DNA polymerase δ and participates in DNA repair and cell division. The proliferative potential of chondrocytes (the ability of cartilage cells to divide) it decreases in osteoarthritis. It leads to a violation of the structure and function of cartilage. The polypeptide complex of cartilage and bone tissues and the AED tripeptide restore PCNA synthesis in chondrocytes and promote cartilage repair in osteoarthritis.

The efficacy of Sigumir® was evaluated on 33 patients (42-59 years old) with osteochondrosis of the lumbar spine. Patients of the control group received conventional treatment. Patients of the main group, in addition to conventional treatment, received Sigumir® 1-2 capsules 2-3 times a day during 30 days. The severity of pain syndrome decreased in 67.4% of patients after the course of treatment with Sigumir®. The greatest effect was obtained in younger patients with the initial stage of the disease. This is due to the fact that the progression of the disease, accompanied by arthrotic changes in the intervertebral discs on the X-ray, contributes to the development of spondylosis and neurotrophic disorders. The positive effect of the polypeptide complex of cartilage and bone tissue was also noted according to radiography.

The application of Sigumir® for 45-60 days in patients with osteoarthritis of the knee joints (7 men, 3 women aged 45 to 78 years) reduces the severity of pain syndrome and increase joint mobility in 68.5% of cases. At the same time, pain symptoms disappeared most completely with radiologically determined initial stages of the disease: narrowing of the articular gap between the patella and hip, lateral osteophytes of the patella and the condyle of the thigh.

The efficacy of Cartalax® was studied in patients (52-72 years old) with osteoarthritis of the knee joints. Cartalax® was used daily for 20 days for 6 capsules per day in addition to conventional treatment in patients over 65 years of age with severe joint deformity. Patients aged 60-65 years old with an average degree of joint deformity were prescribed Cartalax® daily for 20 days, 4 capsules per day.

Cartalax® was used daily for 20 days, 1 capsule 2 times a day in patients aged 52-60 years old with the initial stage of the disease (joint pain). The control group of patients received conventional treatment. The application of Cartalax® reduced the severity of pain syndrome in all groups of patients in 55-63% of cases. At the same time, pain symptoms disappeared most completely with radiologically determined initial stages of the disease: narrowing of the articular gap between the patella and hip, lateral osteophytes of the patella and the condyle of the thigh. There was no significant dynamics of radiological symptoms during this period. At the same time, patients in the advanced stage of arthrosis also had a similar dynamics of subjective indicators, but less pronounced. This stage of the disease was diagnosed in persons of the older age group, therefore, such subjective feelings were characterized as very favorable.

Thus, the polypeptide complex of cartilage and bone tissues (Sigumir®) and its constituent tripeptide AED (Cartalax®) have shown high efficiency in the complex therapy of diseases of the musculoskeletal system (osteoarthritis, osteoporosis, osteochondrosis, etc.), in the experiment and in the clinic. The AED tripeptide normalizes bone density in osteoporosis by regulating the function of calcitonin-producing thyroid cells. The mechanism of action of the polypeptide complex of cartilage and bone tissues and the AED tripeptide is their ability to reduce the synthesis of p53 (protein, which involved in the programmed cell death) and increase the synthesis of PCNA (protein of cell division) in chondrocytes (cartilage cells).