Original article
https://doi.org/10.18019/1028-4427-2024-30-3-406-416

The effect of the IL-6 monoclonal blocker on the course of aseptic femoral head necrosis in the experiment (pilot study)

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Abstract

Background There is currently no pathogenetically based treatment for aseptic necrosis of the femoral head. One of the most promising areas of possible targeted therapy is the use of genetically engineered drugs, including monoclonal blockers of proinflammatory cytokines, aimed at inhibiting inflammation and indirectly reducing the activity of osteodestruction. The aim of the work is to evaluate the effectiveness of the use of the IL-6 monoclonal blocker in the course of aseptic necrosis of the femoral head in an experiment.

Purpose Evaluate the preliminary results of the use of the IL-6 monoclonal blocker in the course of aseptic necrosis of the femoral head in an experiment.

Materials and methods Surgical induction of aseptic necrosis of the femoral head was performed in 18 male Wistar rats. The animals were divided into two groups of 9 individuals each. The first group did not receive any treatment, the second received therapy with a monoclonal IL-6 receptor blocker, starting from the second week of the experiment, one injection once every two weeks. All animals were removed from the experiment at 4, 6 and 8 weeks after the induction of aseptic necrosis, 3 rats from each group at a time. Total RNA was isolated from the femoral head on the aseptic necrosis side and the conditionally healthy side as a control. The expression of genes of regulatory proteins of osteogenesis was studied by PCR. To study the features of osteodestructive processes, histological examination of femoral head preparations in all animals was conducted.

Results Histological preparations of femoral heads of the second group animals were characterized by less pronounced osteodestructive, chondrodestructive processes compared to the animals that did not receive therapy. The mRNA profile of the rats of the second group displayed an increase in the expression of genes encoding proteins involved in osteoreparation at all stages of the experiment. At the same time, the activity of genes encoding proteins of proinflammatory cytokines, regulatory molecules of osteoclastogenesis was reduced relative to the first group.

Discussion The data obtained indicate an important role of inflammation in the regulation of osteodestruction. Inhibition of the biological action of IL-6 contributed to inhibition of the expression of osteoclastogenesis genes, increased activity of bone metabolism genes, and caused a decrease in the intensity of osteodestruction and activation of osteoreparation.

Conclusion Preliminary results of the use of a monoclonal blocker of the proinflammatory cytokine IL-6 indicate the inhibition of osteodestructive and strengthening of osteoreparative processes due to the correction of the expression of bone metabolism genes during the progression of aseptic necrosis of the femoral head in rats in an experimental model.

Keywords: aseptic necrosis, genetically engineered drugs, osteodestruction, IL-6 monoclonal blocker, osteoblastogenesis, osteoclastogenesis, osteoinduction, anti-inflammatory therapy

For citation: Shabaldin NA, Sinitskaya AV, Igisheva LN, Bogdanov LA, Shabaldin AV. The effect of the IL-6 monoclonal blocker on the course of aseptic femoral head necrosis in the experiment (pilot study). *Genij Ortopedii*. 2024;30(3):406-416. doi: 10.18019/1028-4427-2024-30-3-406-416

INTRODUCTION

Aseptic necrosis of the femoral head is a pathological process that develops in stages and in which osteoresorption in the early stages is replaced by activation of osteoreparation in later stages [1, 2, 3]. The result of bone destruction may be the development of deforming coxarthrosis, gross incongruence in the hip joint with the need for its replacement. The pathogenesis of avascular necrosis of the femoral head is not fully understood; however, recently there has been a tendency towards increasing interest in studying disorders of the regulation of bone homeostasis at the molecular and cellular levels [4, 5]. The most commonly used option for conservative therapy in aseptic necrosis of the femoral head is physical therapy aimed at improving blood supply to the proximal femur for enhancing ossification along with compliance with the orthopaedic regimen [6]. This treatment cannot be considered as a targeted treatment and in some cases does not have significant effect. To develop new pathogenetically based strategies for the treatment of osteogenesis disorders, a detailed understanding of the pathophysiological mechanisms of changes in bone tissue metabolism and possible ways of effecting on them is necessary.

The balance of bone tissue remodeling is maintained due to the functioning of a large number of intracellular and intercellular signaling pathways, phosphorylation processes, and the synthesis of regulatory molecules [7, 8]. The main signaling pathway aimed at differentiation and activation of mature osteoclasts is the receptor activator of nuclear factor kappa β, its ligand and osteoprotegerin (RANK-RANKL-OPG) system [9, 10]. The interaction of the membrane receptor activator of nuclear factor kappa β (RANK) with its ligand (RANKL) leads to translocation of nuclear factor kappa β (NFkβ) into the cell nucleus and activation of intracellular pathways, resulting in the induction of differentiation of progenitor cells into a mature osteoclast and subsequent activation. Some research works indicate one of the leading roles of increased osteoclastogenesis in the development of bone destructive processes in avascular necrosis [11, 12].

A number of signaling molecules, including pro-inflammatory cytokines, may be important in regulating RANKL synthesis. The biological effect of pro-inflammatory cytokines, such as IL-6, IL-1β, TNFα, has been proven, aimed at increasing the expression of RANKL, activating osteoclastogenesis and, as a consequence, increasing osteolysis [13, 14, 15]. Moreover, the role of pro-inflammatory cytokines in apoptosis and autophagy of osteoblasts through the mitogen-activated protein kinase (MAPK)/nuclear factor-κB (NF-κB) signaling pathway has been discovered [16]. Regulation of osteoclast activity and, consequently, impact on bone homeostasis is possible through correction of the activity of pro-osteoclastogenic mediators. Thus, a number of research studies reveal an increased concentration of pro-inflammatory cytokines in the synovial fluid in the early stages of avascular femoral head necrosis and consider these regulatory molecules as a possible therapeutic target for the treatment of this disease [17, 18].

A large number of signaling pathways are involved in the regulation of osteoblastogenesis activity: canonical and non-canonical wnt (wingless) / b catenin, JAK (Janus Kinase) / STAT (Signal Transducer and Activator of Transcription), MAPK (mitogen-activated protein kinase) [19, 20, 21]. Along with antiresorption therapy, it is possible to use anabolic therapy, the purpose of which is to enhance osteoinduction in aseptic necrosis of the femoral head. An earlier study of the expression of genes encoding proteins of pro-inflammatory cytokines, molecules involved in osteoclastogenesis, osteoblastogenesis, and bone matrix proteins in rats after surgical induction of aseptic necrosis of the femoral head showed an earlier suppression of the osteogenic component of bone homeostasis than an increase in the osteoresorptive component [22].

Considering the key importance of tissue hypoxia due to hypoperfusion in the development of avascular necrosis of the femoral head, a number of studies link the pathogenesis of osteodestructive processes with overexpression of hypoxia-induced factor 1α (HIF-1α). Thus, a model experiment on piglets resulted in detection of an increased concentration of the HIF-1α protein in the femoral

head during the manifestation of avascular necrosis [23]. It is known that the HIF-1α protein plays an important role in the processes of remodeling and maintenance of bone homeostasis [24, 25, 26]. At the same time, a number of research studies have found an inductive effect of both gene overexpression and the HIF-1α protein itself on the concentration of RANKL and, as a consequence, activation of osteoclast differentiation from progenitor cells [27, 28]. Accordingly, antiresorption therapy aimed at reducing the intensity of osteoclastogenesis would be possible by correcting the activity of HIF-1α as one of the trigger factors in avascular necrosis.

Thus, the regulatory mechanisms that determine the high activity of osteoclasts are being studied in order to develop a targeted therapy aimed at normalizing intercellular interactions. Effective correction of various links in the pathogenesis of osteodestruction would significantly improve the quality of life of patients with aseptic necrosis.

Purpose of the work was to evaluate the preliminary results of the application of the IL-6 monoclonal blocker in the course of aseptic necrosis of the femoral head in an experiment.

MATERIALS AND METHODS

A model experiment was carried out on 18 male Wistar rats, weighing (250 ± 25) g, at the age of three months. To induce aseptic necrosis of the femoral head, all animals underwent surgical manipulations that comprised a dense ligature with absorbable Vicryl suture material around the femoral neck to create a zone of hypoperfusion, as well as introducing 1.5 ml of a 2 % rheopolyglucin solution into the joint cavity to increase intra-articular pressure. The model of induction of aseptic femoral head necrosis is protected by a patent of the Russian Federation [29].

The animals are then divided into two equal groups. Animals of the first group did not receive treatment (comparison group). Animals of the second group received an injection of sarilumab (kevzara), a monoclonal blocker of the IL-6 receptor, starting from the second week of the experiment, one injection once every two weeks, at a dose of 15 mg per kg of body weight (study group). They were kept in cages of three animals with free access to food and water. All animals were removed from the experiment by decapitation at 4, 6 and 8 weeks after the induction of aseptic necrosis, three rats from each group. The experiment was carried out in accordance with the "Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" adopted by the Council of Europe (Strasbourg, France, 1986), and Council Directive 86/609/EEC of 24.11.1986 "On the harmonization of laws, regulations and administrative orders of the participating countries regarding the protection of animals used for experimental and scientific purposes" on the basis of the Kemerovo State Medical University and the Research Institute of the Communist Party of the Soviet Union (Kemerovo).

After removing the animals from the experiment, extirpation of the femurs was performed, both from the side of aseptic necrosis manifestation and from a conditionally healthy side as a comparison. The proximal epiphysis was separated from the femur and divided into two equal parts. A part of the femoral head was used for histological examination. The specimen was prepared with a standard acid-free method using EDTA salts and stained with hematoxylin and eosin. To evaluate the morphometric parameters, ImageJ software was used to identify the colored bone beams as a pixel value, which enabled to quantify the morphometric parameters.

A part of the femoral head, both affected by aseptic necrosis and a healthy one, was used to isolate total RNA using the RNeasy MicroKit kit (QIAGEN, Germany) according to the manufacturer's protocol. The quality and quantity of isolated RNA was determined on a Qubit 4 spectrophotometer (Invitrogen, USA) by assessing the RIQ index (RNA Integrity and Quality) using the Qubit RNA IQ Assay Kit (Invitrogen, USA).

Gene expression levels were determined by quantitative reverse transcription polymerase reaction using the High-Capacity cDNA Reverse Transcription Kit (4368814, Thermo Fisher Scientific,

Waltham, MA, USA). Primers were synthesized on an ABI 3900 high-throughput DNA synthesiser (Thermo Fisher Scientific, Waltham, MA, USA) by Evrogen (Moscow, Russia). Results of qPCR were normalized using three reference genes *actb*, *tbp*, *b2m* in accordance with existing recommendations. The expression of the studied genes was calculated using the 2-ΔΔCt method. Gene expression was studied for *il4*, *il6*, *il1b*, *tnfa*, *tgfb*, *sp7*, *runx2*, *opn/spp1*, *bmp2*, *bglap*, *rankl*, *alpl*, *hif1a*.

Statistical processing of the obtained results was carried out in Statistica for WINDOWS software packages from StatSoftInc (USA), version 10.0 according to the rules of variation statistics. The study used Wistar rats kept under the same optimal conditions, so the Shapiro – Wilk W-test showed a normal distribution. Quantitative data were presented as mean and standard error (M ± m). Comparison of the values of metric indicator levels in unrelated samples was carried out using the Student's t test. The probability of a type 1 error was taken as 5 %, and a second type error as 20 %; accordingly, the level of statistical significance was detected at $p < 0.05$, which corresponds to standard requirements. A pure line of Wistar laboratory rats was used in the experiment, which determined the genetic homogeneity of the studied groups. Thus, the study of even a small sample enabled statistical processing of the results obtained.

RESULTS

Histological study

The motor activity of the rats that received injections of a monoclonal IL-6 blocker recovered faster than in the comparison group. Thus, all rats of the study group were able to stand on their hind legs 4 weeks after induction of aseptic necrosis, while in the comparison group only a part of the animals could bear weight on the pelvic limbs at 6 weeks after the start of the experiment (Fig. 1).

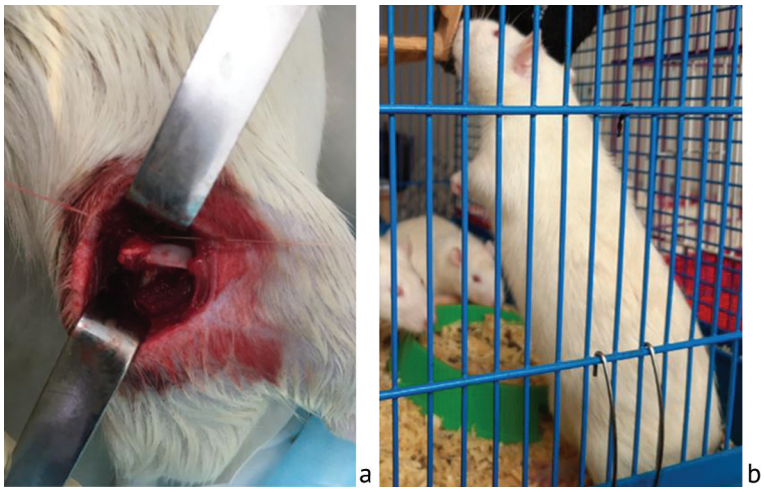

Fig. 1 Photos of the experiment: *a* surgical induction of aseptic necrosis of the femoral head; *b* axial load on the hind legs in rats of the study group

The histological picture of preparations of the femoral heads in both groups confirmed the development of chondrodestructive and osteodestructive processes. The study revealed empty bone lacunae; part of the bone tissue was replaced by dense fibrous tissue. In the areas of osteoresorption, active osteoclasts were identified as giant multinucleated cells. The number of osteoblasts, which were defined as mononuclear cells, increased primarily in the areas of osteosclerosis. However, the course of avascular necrosis differed. Progressive osteoresorption from the fourth to the sixth week was noted in animals of the first group, while signs of osteoreparation were visualized only in the eighth week of the experiment. In the group of animals receiving the monoclonal IL-6 blocker drug, isolated osteolytic processes were recorded only in the fourth week; active osteoblasts, areas of revascularization and bone restoration were visualized in the sixth and eighth weeks along with osteodestruction.

Four weeks after surgical induction of aseptic necrosis, a change in the normal architecture of the bone trabeculae of the cancellous substance to a wave-like arrangement was visualized in the rats that did not receive treatment, and the density of trabeculae was reduced. The intermediate layer of yaline cartilage was characterized by sparseness; most of the chondrocytes were located in the outer layer. Chondrocytes lost their elliptical shape, and signs of destruction of their nucleus were

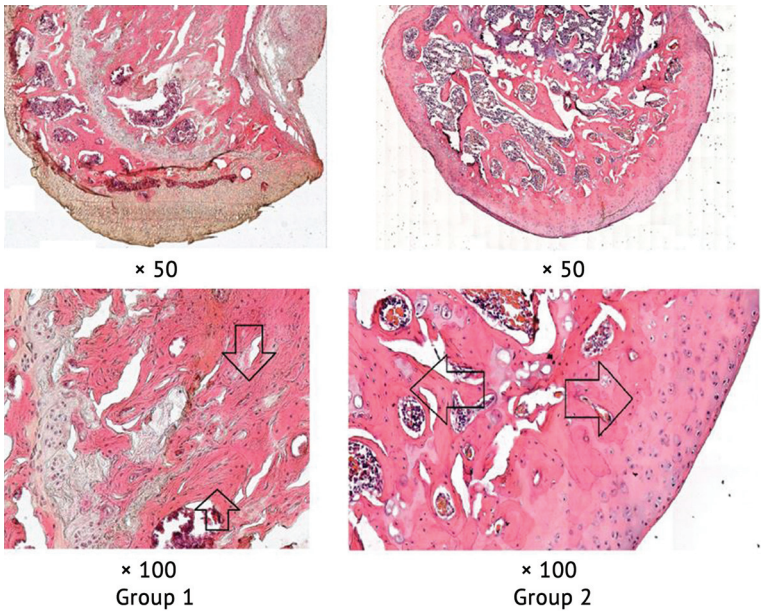

Fig. 2 Histological picture at 4 weeks after the manifestation of aseptic necrosis (hematoxylin and eosin staining). Group 1: arrows show the wavy position of bone trabeculae; group 2: arrows show a uniform arrangement of chondrocytes, normal architecture of the spongy substance

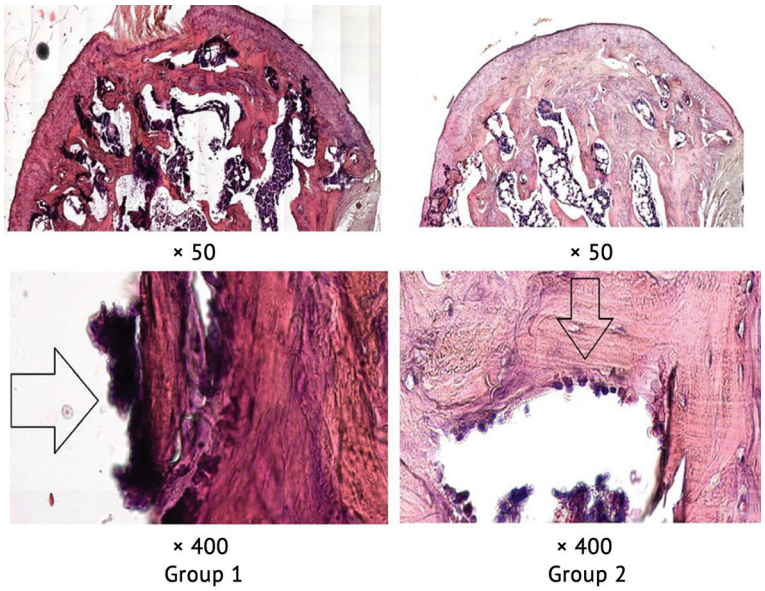

Fig. 3 Histological picture at 6 weeks after the manifestation of aseptic necrosis (hematoxylin and eosin staining). Group 1: arrows show active osteoclasts; group 2; arrows show active osteoblasts

revealed. In animals of the second group, preparations of the femoral heads largely preserved normal architecture, and the loss of bone trabeculae was lower than in rats of the first group. The arrangement of chondrocytes in hyaline cartilage was more uniform (Fig. 2).

In preparations of the femoral heads bones of the first group animals, six weeks after the start of the experiment, signs of progressive osteoresorption were observed, and a further loss of bone trabeculae density was diagnosed in comparison with preparations taken after four weeks of the aseptic necrosis modeling. The number of active osteoclasts and bone resorption lacunae increased from the fourth to the sixth week of the experiment. Part of the hyaline cartilage was replaced by fibrous tissue. In the animals of the second group, active osteoblasts were determined in the areas of bone sclerosis along with signs of osteodestruction in the form of loss of a part of the bone trabeculae and their wavy arrangement in the preparations of the femoral heads. At the same time, the loss of bone trabeculae density in the animals treated with a monoclonal IL-6 blocker was lower than in rats of the first group (Fig. 3).

After 8 weeks, the first signs of osteogenicity were noted in the femoral heads of rats that did not receive treatment. Thinning of the articular cartilage, loss of some chondrocytes, and bone trabeculae were visualized. At the same time, along with replacement of the spongy substance with dense fibrous tissue, active osteoblasts were determined. In the rats of the second group, hyaline cartilage was characterized by greater preservation of the density of chondrocytes in both the outer and intermediate layers. Bone trabeculae had a high density, zones of mineralization with a large accumulation of active osteoblasts, and areas of bone revascularization were identified. The number of osteocytes increased compared to the preparations taken 6 weeks after the induction of aseptic necrosis (Fig. 4).

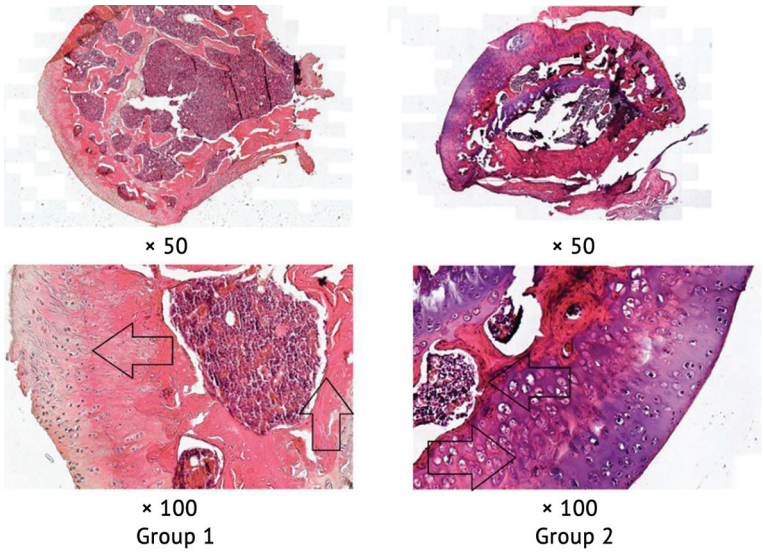

Fig. 4 Histological picture at 8 weeks after the manifestation of aseptic necrosis (hematoxylin and eosin staining). Group 1: arrows show uneven distribution of chondrocytes in hyaline cartilage, active osteoblasts; group 2: arrows show a uniform, dense distribution of chondrocytes in hyaline cartilage and an area of bone mineralization

The use of ImageJ software allowed us to quantify changes in bone trabecular density. Thus, in the rats that did not receive treatment, the course of avascular necrosis was accompanied by a rogressive loss of bone density from the fourth to the sixth week. At the same time, in the eighth week of the experiment, a slight increase in the density of the bone trabeculae was noted. A tendency towards a decrease in the density of bone trabeculae from the fourth to the sixth week and recovery in the eighth week of the experiment was also recorded in the study group. However, the volume of bone trabeculae in the animals that received anti-inflammatory therapy with a monoclonal IL-6 blocker was significantly greater than in the rats without treatment at the sixth and eighth weeks after the induction of avascular necrosis (Table 1).

Table 1

Histological findings of trabecular volume (%)

Week of experiment	Comparison group	Study group	*p*-level
4	31.45 ± 1.03	33.67 ± 1.43	> 0.05
6	23.03 ± 1.23	30.78 ± 1.32	0.02*
8	24.43 ± 1.29	31.66 ± 1.36	0.03*

Note: * — significant difference

Changes in mRNA profile

Features of the functioning of signaling pathways for bone tissue remodeling and their regulation at the molecular and cell level determined the activity of osteoresorptive and osteoreparative processes. One month after the surgical induction of avascular necrosis, the greatest differences were obtained in the dynamics of changes in genes encoding proteins associated with osteoinduction. Thus, in animals of the second group along with a decrease in the expression of the gene for the pro-inflammatory cytokine interleukin 6 (*il6*), the expression of the osteocalcin gene (*bglap*), the encoded protein of which is secreted by osteoblasts and is involved in the regulation of bone remodeling, and the alkaline phosphatase gene (*alpl*), which determines the intensity of bone mineralization, increased significantly , the transforming growth factor b (*tgfb*) gene, the encoded protein of which enhances revascularization and is involved in osteoreparation processes. Moreover, in rats treated with a monoclonal IL-6 blocker, the expression of the hypoxia-induced factor 1α (*hif1α*) gene was significantly reduced. In the animals of the first group, the expression of the secreted phosphoprotein 1 (*spp1*) gene increased significantly. The protein encoded by this gene promotes the adhesion of osteoclasts to the bone matrix and has high specificity for hydroxyapatite and osteoclast membrane proteins (Table 2).

Six weeks after the induction of aseptic necrosis of the femoral head, overexpression of the proinflammatory cytokine genes *il6*, *tnfα*, as well as the nuclear factor κβ (*rankl*) receptor activator ligand gene was observed in the rats that did not receive treatment (comparison group). This gene encodes one of the main proteins of the RANK–RANKL–OPG signaling pathway, aimed at the differentiation and activation of osteoclasts. At the same time, in the animals treated with a monoclonal IL6 blocker, the expression of the *il6*, *tnfα* gene was significantly suppressed. The intensity of *rankl* gene expression did not differ from the conditionally healthy limb. Also, in rats of the main group, increased expression of genes aimed at enhancing osteoblastogenesis was recorded, such as the bone morphogenetic protein gene (*bmp2*), the transcription factor gene (*sp7*), involved in osteoblast differentiation, and the expression of *tgfb* genes remained increased. However, it is worth noting the increased expression of the *spp1* gene, which may indicate osteoclast activity (Table 3).

Two months after the development of avascular necrosis of the femoral head, overexpression of the *il6*, *tnfα* genes was retained in the rats of the first group. The intensity of *rankl* gene expression decreased slightly compared to the previous period, but was higher than in a conditionally healthy limb. The synthesis of *spp1* mRNA gene increased. However, changes in the mRNA profile in the femoral heads of the untreated animals also indicate increased osteogenesis. The expression of *alpl* genes increased. In the rats of the second group, an increase in the expression of genes encoding proteins was retained, the biological action of which is aimed at enhancing osteoreparation. The synthesis of the mRNA genes *runx2* and *sp7* remained increased. However, against this background, the intensity of *rankl* gene expression was increased compared to a conditionally healthy limb (Table 4).

Table 2

mRNA profile after 4 weeks

Gene	Comparison group	Study group	*p*-level
bmp2	1.097 ± 0.219	0.831 ± 0.137	> 0.05
alpl	0.484 ± 0.095	2.445 ± 0.738	0.02*
hif1a	1.031 ± 0.563	0.342 ± 0.059	0.04*
rankl	1.216 ± 0.609	1.157 ± 0.583	> 0.05
runx2	0.934 ± 0.319	0.891 ± 0.204	> 0.05
sp7	1.051 ± 0.421	1.774 ± 0.538	> 0.05
bglap	0.684 ± 0.137	3.637 ± 0.926	0.01*
spp1	7.691 ± 1.823	0.624 ± 0.137	< 0.001*
tgfb	0.964 ± 0.371	3.092 ± 0.957	0.01*
tnfa	1.142 ± 0.296	0.806 ± 0.172	> 0.05
il6	1.125 ± 0.325	0.79 ± 0.148	0.04*

Note: * — significant difference.

Table 3

mRNA profile after 6 weeks

Gene	Comparison group	Study group	*p*-level
bmp2	1.613 ± 0.419	2.462 ± 0.816	0.03*
alpl	2.394 ± 0.751	2.936 ± 0.973	> 0.05
hif1a	0.768 ± 0.143	1.287 ± 0.419	> 0.05
rankl	11.076 ± 3.054	1.019 ± 0.326	0.001*
runx2	3.831 ± 0.904	0.501 ± 0.118	0.01*
sp7	1.998 ± 0.673	3.095 ± 1.008	0.04*
bglap	0.702 ± 0.179	1.043 ± 0.307	> 0.05
spp1	0.447 ± 0.103	2.297 ± 0.713	0.01*
tgfb	0.943 ± 0.319	2.331 ± 0.784	0.02*
tnfa	14.471 ± 4.107	1.918 ± 0.607	< 0.001*
il6	3.844 ± 0.916	0.054 ± 0.014	< 0.001*

Note: * — significant difference.

Table 4

mRNA profile after 8 weeks

Gene	Comparison group	Study group	*p*-level
bmp2	1.613 ± 0.419	2.462 ± 0.816	0.03*
alpl	2.394 ± 0.751	2.936 ± 0.973	> 0.05
hif1a	0.768 ± 0.143	1.287 ± 0.419	> 0.05
rankl	11.076 ± 3.054	1.019 ± 0.326	0.001*
runx2	3.831 ± 0.904	0.501 ± 0.118	0.01*
sp7	1.998 ± 0.673	3.095 ± 1.008	0.04*
bglap	0.702 ± 0.179	1.043 ± 0.307	> 0.05
spp1	0.447 ± 0.103	2.297 ± 0.713	0.01*
tgfb	0.943 ± 0.319	2.331 ± 0.784	0.02*
tnfa	14.471 ± 4.107	1.918 ± 0.607	< 0.001*
il6	3.844 ± 0.916	0.054 ± 0.014	< 0.001*

Note: * — significant difference.

DISCUSSION

Changes in the mRNA profile during the progression of aseptic femoral head necrosis are characterized by extreme heterogeneity. At the same time, some patterns of synthesis of genes encoding regulatory proteins of osteogenesis can determine the course of osteodestructive and osteoreparative processes.

The development of avascular necrosis in the animals that did not receive therapy, according to histological examination, was accompanied by a progenitor course of osteoresorptive and chondrodestructive processes up to 8 weeks after the hypoperfusion zone of the femoral head had been applied. Only eight weeks after the start of the experiment, the first signs of osteoreparation were detected in histological preparations.

At the same time, changes in the mRNA profile of the proximal femoral epiphysis show a tendency toward overexpression of proinflammatory cytokine genes and osteoclastogenesis genes from the fourth to the eighth week. Thus, increased expression of the *il6, tnfα, rankl* genes was observed both in the sixth and eighth weeks of aseptic necrosis. These genes encode proteins of pro-inflammatory cytokines and key molecules of the osteoclastogenesis signaling pathway. Song et al. in their study of the proteome in patients with aseptic necrosis of the femoral head noted an increase in the concentration of pro-inflammatory cytokines and attribute great importance to the increase in nonspecific inflammation in the processes of dysregulation of bone homeostasis [30]. The biological effect of proinflammatory cytokines is aimed at inducing the RANK–RANKL–OPG signaling pathway, enhancing the differentiation and activation of mature osteoclasts. There are a number of research studies confirming the overexpression of these inflammatory mediators in the early stages of the development of aseptic femoral head necrosis [31, 32]. Thus, it is logical to consider the relationship between an increase in the level of mRNA in the head of the femur of both the proinflammatory cytokine genes *il6, tnfα*, and the *rankl* gene, and the histological picture of the progressive destruction o bone and cartilage tissue. The data obtained indicate the important role of inflammation in the regulation of osteodestruction.

Moreover, at the fourth and eighth weeks after surgical induction of avascular necrosis, overexpression of the *spp1* gene was observed. Studies of the concentration of secreted phosphoprotein 1 (*spp1*) in osteoblasts due to the development of avascular necrosis of the femoral head showed a significant increase in the concentration of this protein compared with a conditionally healthy limb [33]. It is known that the encoded protein of the *spp1* gene promotes the attachment of osteoclasts to the bone matrix [34]. Besides, an increase in *spp1* gene expression may promote the activation of osteoclastogenesis through the PI3K/AKT signaling pathway [35]. Thus, an increase in the expression of the gene in the histological picture of progressive osteodestruction from the fourth to the eighth week of the experiment can also be considered as one of the factors contributing to the intensification of resorption processes.

However, in the eighth week of the experiment, a large number of active osteoblasts were determined by examining histological preparations of the animals from the first group. At the same time, the expression of genes encoding proteins involved in bone mineralization and osteoblastogenesis (*bmp2, alpl, bglap*) increased. Thus, the processes of regeneration and destruction of bone tissue are largely due to the changes in the mRNA profile of the femoral head.

Changes in the study of histological preparations of the femoral head in the rats treated with a monoclonal blocker of IL-6 were characterized by a less intense course of osteodestruction compared to the animals of the first group. The density of bone trabeculae, the architectonics of the spongy substance, and changes in the hyaline cartilage were less pronounced and more consistent with findings in the conditionally healthy limb. Moreover, already from the sixth week after surgical induction of avascular necrosis, signs of osteoreparation were visualized in the preparations.

The dynamics of changes in the mRNA profile of genes encoding regulatory proteins of osteogenesis had its own characteristics. Expression of the gene for the pro-inflammatory cytokine *il6* was expectedly reduced in the fourth and sixth weeks of the experiment. Maximum inhibition of *il6* gene activity was obtained in the sixth week, while a slight increase in the expression was observed in the eighth week. The activity of the *tnfα* gene varied depending on the time after the manifestation of aseptic necrosis. Thus, the expression of the *tnfα* gene in the fourth week of the experiment was slightly reduced, while in the sixth and eighth weeks it was increased compared to a conditionally healthy limb. It is worth noting that the activity of the pro-inflammatory cytokine genes *il6, tnfα* at all stages of the experiment in the rats of the second group was significantly lower than in the first group. The presented data indicate inhibition of nonspecific inflammation due to the therapy with a monoclonal IL-6 blocker.

A decrease in the intensity of the biological action of the pro-inflammatory cytokine IL-6 can be associated with changes in the expression of genes encoding proteins that regulate osteoclastogenesis and the activity of osteolysis processes. The most striking picture of osteoclastogenesis inhibition was observed at week 6 of the experiment. Thus, in the animals that did not receive specific treatment, overexpression of the *rankl* gene was observed, while in the rats treated with a monoclonal blocker of IL-6, the expression of the gene did not differ from that of a conditionally healthy limb. However, at the same time, in the rats of the second group, an increase in the expression of the *spp1* gene was recorded, which may indicate processes of osteolysis and, possibly, indirect pathways of activation of mature osteoclasts.

The first four weeks after the induction of avascular necrosis in the animals of the second group were accompanied by increased expression of genes associated with osteoinduction and revascularization, such as *sp7, bglap, alpl, tgf*β. The predominance of the expression of the genes encoding proteins of osteoblastogenesis, extracellular bone matrix, and bone mineralization was maintained from the fourth to the eighth week of the experiment, while the nature of the mRNA profile somewhat changed in regard to the time elapsed since the manifestation of aseptic necrosis of the femoral head. There are a number of research studies that reveal the effectiveness of antiresorptive, anti-inflammatory therapy in experimental model of aseptic necrosis of the femoral head in animals [31, 32, 36]. The effectiveness of treatment was assessed using data from microcomputed tomography, histological, and immunohistochemical studies, which showed an increase in bone tissue repair processes and a decrease in osteoresorption compared to a conditionally healthy limb. However, studies of the molecular and cellular mechanisms of osteogenesis regulation during the use of monoclonal blockers of proinflammatory cytokines are very few.

The intensity of expression of the *sp7* transcription factor gene was increased throughout the experiment in the rats of the second group. The protein encoded by this gene is a member of the zinc factor family of transcription factors, which play a fundamental role in the processes of differentiation of osteoblasts from progenitor cells, chondrogenesis, as well as maintaining the balance in the differentiation of mesenchymal cells along the osteogenic and chondrogenic pathway [37]. Increased expression of the *sp7* gene in the fourth, sixth and eighth weeks after the induction of avascular necrosis by administration of a monoclonal IL-6 blocker may indicate increased chondrogenesis and osteogenesis. Moreover, in the rats of the second group, the expression of the alkaline phosphatase gene *alpl* was increased from the fourth to the eighth week of the experiment. These findings confirm an increase in bone tissue metabolism along with suppression of the biological effect of the pro-inflammatory cytokine IL-6.

Thus, the preliminary results of the use of a monoclonal blocker of the proinflammatory cytokine IL-6 show a more favorable course of aseptic necrosis of the femoral head in rats. Greater preservation of the density of bone trabeculae, the structure of hyaline cartilage, and cancellous bone was obtained in the animals that received specific anti-inflammatory therapy. The dynamics of changes in the mRNA profile in the rats of the study group indicate inhibition of the expression of osteoclastogenesis and osteolysis genes (*rankl, spp1*) due to a decrease in the activity of the proinflammatory cytokine genes *il6, tnf*α. At the same time, the expression of genes encoding regulatory proteins of osteoblastogenesis and bone tissue mineralization was increased at all stages of the experiment. However, it is worth noting that the results obtained are relevant in the case of early administration of the specific anti-inflammatory therapy with genetically engineered drugs, before the stage of femoral head fragmentation. Moreober, some regulatory proteins are synthesized at the post-translational level. Further research with a larger sample size will allow us to draw more reliable conclusions about the effectiveness of anti-inflammatory therapy in correcting bone homeostasis. Also, the study of the methods of targeted therapy and of the pathogenesis of avascular necrosis should assess the concentration of regulatory proteins involved in osteogenesis and bone tissue remodeling processes.

CONCLUSION

The preliminary results of the application of a monoclonal blocker of the pro-inflammatory cytokine IL-6 indicate inhibition of osteodestructive and enhancement of osteoreparative processes by correcting the expression of bone metabolism genes in the course of aseptic necrosis of the rat's femoral head in an experimental model.

Conflict of interest *The authors declare that there are no obvious or potential conflicts of interest related to the publication of this article.*

Funding *The study was carried out with funding from the grant "President of the Russian Federation for state support of young Russian scientists — candidates of science", MK-4132.2022.3.*

Ethical statement *The study was approved by the ethics committee of the Federal State Budgetary Educational Institution of Higher Education Kemerovo State Medical University, protocol No. 1 dated January 20, 2023.*

REFERENCES

1. Kuzhelivsky AI. Perthes disease: the current state of the problem and ways to solve it. *Siberian Medical Journal* (Irkutsk). 2015;135(4):117-121. (In Russ.)
2. Hines JT, Jo WL, Cui Q, et al. Osteonecrosis of the Femoral Head: an Updated Review of ARCO on Pathogenesis, Staging and Treatment. *J Korean Med Sci*. 2021;36(24):e177. doi: 10.3346/jkms.2021.36.e177
3. Mukisi-Mukaza M, Gomez-Brouchet A, Donkerwolcke M, et al. Histopathology of aseptic necrosis of the femoral head in sickle cell disease. *Int Orthop*. 2011;35(8):1145-1150. doi: 10.1007/s00264-010-1121-6
4. Adapala NS, Kim HK. Comprehensive Genome-Wide Transcriptomic Analysis of Immature Articular Cartilage following Ischemic Osteonecrosis of the Femoral Head in Piglets. *PLoS One*. 2016;11(4):e0153174. doi: 10.1371/journal.pone.0153174
5. Naik AA, Narayanan A, Khanchandani P, et al. Systems analysis of avascular necrosis of femoral head using integrative data analysis and literature mining delineates pathways associated with disease. *Sci Rep*. 2020;10(1):18099. doi: 10.1038/s41598-020-75197-0
6. Odarchenko DI, Dzyuba GG, Erofeev SA, Kuznetsov NK. Problems of diagnosis and treatment of aseptic necrosis of the femoral head in contemporary traumatology and orthopedics (literature review). *Genij Ortopedii*. 2021;27(2):270-276. doi: 10.18019/1028-4427-2021-27-2-270-276
7. Tang CY, Wu M, Zhao D, et al. Runx1 is a central regulator of osteogenesis for bone homeostasis by orchestrating BMP and WNT signaling pathways. *PLoS Genet*. 2021;17(1):e1009233. doi: 10.1371/journal.pgen.1009233
8. Li Q, Wang Z. Involvement of FAK/P38 Signaling Pathways in Mediating the Enhanced Osteogenesis Induced by Nano-Graphene Oxide Modification on Titanium Implant Surface. *Int J Nanomedicine*. 2020;15:4659-4676. doi: 10.2147/IJN.S245608
9. Udagawa N, Koide M, Nakamura M, et al. Osteoclast differentiation by RANKL and OPG signaling pathways. *J Bone Miner Metab*. 2021;39(1):19-26. doi: 10.1007/s00774-020-01162-6
10. Gerstein EU, Timofeev YuS, Zuev AA, Kushlinsky NE. RANK/RANKL/OPG ligand-receptor system and its role in primary bone neoplasms (literature analysis and own data). *Advances in molecular oncology*. 2015;2(3)51-59. (In Russ.) doi: 10.17650/2313-805X-2015-2-3-51-59
11. Samara S, Dailiana Z, Chassanidis C, et al. Expression profile of osteoprotegerin, RANK and RANKL genes in the femoral head of patients with avascular necrosis. *Exp Mol Pathol*. 2014;96(1):9-14. doi: 10.1016/j.yexmp.2013.10.014
12. Chen B, Du Z, Dong X, et al. Association of Variant Interactions in RANK, RANKL, OPG, TRAF6, and NFATC1 Genes with the Development of Osteonecrosis of the Femoral Head. *DNA Cell Biol*. 2019;38(7):734-746. doi: 10.1089/dna.2019.4710
13. Amarasekara DS, Yun H, Kim S, et al. Regulation of Osteoclast Differentiation by Cytokine Networks. *Immune Netw*. 2018;18(1):e8. doi: 10.4110/in.2018.18.e8
14. Zupan J, Jeras M, Marc J. Osteoimmunology and the influence of pro-inflammatory cytokines on osteoclasts. Biochem Med (Zagreb). 2013;23(1):43-63. doi: 10.11613/bm.2013.007
15. Moon SJ, Ahn IE, Jung H, et al. Temporal differential effects of proinflammatory cytokines on osteoclastogenesis. *Int J Mol Med*. 2013;31(4):769-777. doi: 10.3892/ijmm.2013.1269
16. Zheng LW, Wang WC, Mao XZ, et al. TNF-α regulates the early development of avascular necrosis of the femoral head by mediating osteoblast autophagy and apoptosis via the p38 MAPK/NF-κB signaling pathway. *Cell Biol Int*. 2020;44(9):1881-1889. doi: 10.1002/cbin.11394
17. Kamiya N, Kim HK. Elevation of Proinflammatory Cytokine HMGB1 in the Synovial Fluid of Patients With Legg-Calvé-Perthes Disease and Correlation With IL-6. *JBMR Plus*. 2020;5(2):e10429. doi: 10.1002/jbm4.10429
18. Adapala NS, Yamaguchi R, Phipps M, et al. Necrotic Bone Stimulates Proinflammatory Responses in Macrophages through the Activation of Toll-Like Receptor 4. *Am J Pathol*. 2016;186(11):2987-2999. doi: 10.1016/j.ajpath.2016.06.024
19. Ren R, Guo J, Chen Y, et al. The role of Ca2+ /Calcineurin/NFAT signalling pathway in osteoblastogenesis. *Cell Prolif*. 2021;54(11):e13122. doi: 10.1111/cpr.13122
20. Amarasekara DS, Kim S, Rho J. Regulation of Osteoblast Differentiation by Cytokine Networks. *Int J Mol Sci*. 2021;22(6):2851. doi: 10.3390/ijms22062851
21. Eda H, Shimada H, Beidler DR, Monahan JB. Proinflammatory cytokines, IL-1β and TNF-α, induce expression of interleukin-34 mRNA via JNK- and p44/42 MAPK-NF-κB pathway but not p38 pathway in osteoblasts. *Rheumatol Int*. 2011;31(11):1525-1530. doi: 10.1007/s00296-010-1688-7
22. Shabaldin NA, Sinitskaya AV, Shabaldin AV, Mukhamadiyarov RA. Expression Dynamics of Bone Homeostasis Genes in the Development of Aseptic Femoral Head Necrosis in Rats. *J Evol Biochem Phys*. 2023;59(1):180-191. doi: 10.1134/S0022093023010155

23. Yamaguchi R, Kamiya N, Adapala NS, et al. HIF-1-Dependent IL-6 Activation in Articular Chondrocytes Initiating Synovitis in Femoral Head Ischemic Osteonecrosis. *J Bone Joint Surg Am*. 2016;98(13):1122-1131. doi: 10.2106/JBJS.15.01209
24. Yellowley CE, Genetos DC. Hypoxia Signaling in the Skeleton: Implications for Bone Health. *Curr Osteoporos Rep*. 2019;17(1):26-35. doi: 10.1007/s11914-019-00500-6
25. Stegen S, van Gastel N, Eelen G, et al. HIF-1α Promotes Glutamine-Mediated Redox Homeostasis and Glycogen-Dependent Bioenergetics to Support Postimplantation Bone Cell Survival. *Cell Metab*. 2016;23(2):265-279. doi: 10.1016/j.cmet.2016.01.002
26. Ding H, Gao YS, Hu C, et al. HIF-1α transgenic bone marrow cells can promote tissue repair in cases of corticosteroid-induced osteonecrosis of the femoral head in rabbits. *PLoS One*. 2013;8(5):e63628. doi: 10.1371/journal.pone.0063628
27. Zhu J, Tang Y, Wu Q, et al. HIF-1α facilitates osteocyte-mediated osteoclastogenesis by activating JAK2/STAT3 pathway in vitro. *J Cell Physiol*. 2019;234(11):21182-21192. doi: 10.1002/jcp.28721
28. Hirai K, Furusho H, Hirota K, Sasaki H. Activation of hypoxia-inducible factor 1 attenuates periapical inflammation and bone loss. *Int J Oral Sci*. 2018;10(2):12. doi: 10.1038/s41368-018-0015-0
29. Shabaldin N.A., Shabaldin A.V., Shabaldina E.V., et al. *A method for modeling aseptic necrosis of the femoral head in laboratory rats*. Patent RF, no. 2773606, 2022. Available at: https://www.fips.ru/registers-doc-view/fips_servlet. Accessed Jan 16, 2024.
30. Song J, Wu J, Poulet B, et al. Proteomics analysis of hip articular cartilage identifies differentially expressed proteins associated with osteonecrosis of the femoral head. *Osteoarthritis Cartilage*. 2021;29(7):1081-1092. doi: 10.1016/j.joca.2021.04.005
31. Kuroyanagi G, Adapala NS, Yamaguchi R, et al. Interleukin-6 deletion stimulates revascularization and new bone formation following ischemic osteonecrosis in a murine model. *Bone*. 2018;116:221-231. doi: 10.1016/j.bone.2018.08.011
32. Ren Y, Deng Z, Gokani V, et al. Anti-Interleukin-6 Therapy Decreases Hip Synovitis and Bone Resorption and Increases Bone Formation Following Ischemic Osteonecrosis of the Femoral Head. *J Bone Miner Res*. 2021;36(2):357-368. doi: 10.1002/jbmr.4191
33. Maestro-Paramio L, García-Rey E, Bensiamar F, Saldaña L. Osteoblast function in patients with idiopathic osteonecrosis of the femoral head : implications for a possible novel therapy. *Bone Joint Res*. 2021;10(9):619-628. doi: 10.1302/2046-3758.109.BJR-2021-0016.R1
34. Foster BL, Ao M, Salmon CR, et al. Osteopontin regulates dentin and alveolar bone development and mineralization. *Bone*. 2018;107:196-207. doi: 10.1016/j.bone.2017.12.004
35. Cai X, Zheng Y, Ren F, et al. Secretory phosphoprotein 1 secreted by fibroblast-like synoviocytes promotes osteoclasts formation via PI3K/AKT signaling in collagen-induced arthritis. *Biomed Pharmacother*. 2022;155:113687. doi: 10.1016/j.biopha.2022.113687
36. Kim HK, Aruwajoye O, Du J, Kamiya N. Local administration of bone morphogenetic protein-2 and bisphosphonate during non-weight-bearing treatment of ischemic osteonecrosis of the femoral head: an experimental investigation in immature pigs. J *Bone Joint Surg Am*. 2014;96(18):1515-1524. doi: 10.2106/JBJS.M.01361
37. Fiscaletti M, Biggin A, Bennetts B, et al. Novel variant in Sp7/Osx associated with recessive osteogenesis imperfecta with bone fragility and hearing impairment. *Bone*. 2018;110:66-75. doi: 10.1016/j.bone.2018.01.031

The article was submitted 10.08.2023; approved after reviewing 29.11.2023; accepted for publication 08.04.2024.

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