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Effect of allogenic biomaterial on the development of adjuvant arthritis in mature female rats

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Abstract

The administration of dispersed allogeneic biomaterial (AB) into the para-articular region and joint cavity allows slowing down the processes of tissue destruction in arthritis. The aim of the study was to examine the effect of AB on the course of experimental adjuvant arthritis (AA) in rats. For modeling of AA, complete Freund's adjuvant was injected into the plantar surface of the hind paw of 60 white outbred female rats. The study included intact group, control group, and experimental group. After 37 days of the experiment, blood was collected for hematological analysis and the knee joint with surrounding tissues was harvested for standard histological examination. Intra-articular administration of AB to experimental rats while using complete Freund's adjuvant neutralized the manifestation of signs of a generalized inflammatory process in the joints and reduced the degree of destructive changes in the articular apparatus, preserving the structure of the cartilaginous layer. The use of AB made it possible to stabilize the red and white blood cells levels in the experimental group, as well as significantly increase the reduced level of monocytes. Intra-articular administration of AB during AA modeling exhibits an osteo- and chondro-protective effect, providing positive anti-inflammatory and symptom-modifying effects and weakening the manifestation of pathomorphological changes in the joints of experimental rats.

Introduction

Currently, methods of pathogenetic therapy for chondro- and osteo-destructive joint diseases, including rheumatoid arthritis, are being quite widely introduced into the medical practice.[1,2] Such treatment methods are based on restoring the regenerative potential of joint tissues at the site of damage development.[3,4] Allogeneic biomaterials (ABs) for joint diseases represent one of these methods, facilitating the development of effective treatments.[5,6] These materials, obtained from donor tissues, demonstrate unique properties that allow slowing the progression of inflammatory processes and restoring damaged joints.[5,6]

The use of ABs, such as cartilage grafts and osteoblastic cell cultures, can significantly expand the arsenal of pharmacotherapeutic treatment methods.[7,8] These materials can not only replace damaged joint areas but also stimulate tissue regeneration, helping to restore function and reduce pain.[7,8]

Scientific studies showed that The use of ABs significantly helps to increase the effectiveness of traditional treatment methods, such as drug therapy and physiotherapy.[9,10] It is important to note that active work is currently underway to improve the biocompatibility and reduce the immune response to these materials, which will make their use even more widespread and effective in future.[10,11]

The method of introducing dispersed AB into the para-articular region and joint cavity allows slowing down the processes of tissue destruction in arthritis.[5,6] The mechanism of AB action on tissues and processes in a living organism during arthritis has not been fully disclosed.[12-15] Today, the recognized experimental model of rheumatoid arthritis is adjuvant arthritis (AA), which is modeled using complete Freund's adjuvant (CFA), a water-in-oil emulsion containing thermally inactivated *Mycobacterium tuberculosis*.[16] The purpose of this study was to examine the effect of AB on the course of experimental AA in rats.

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Materials and Methods

To model AA, 100 µL of CFA manufactured by Sigma-Aldrich, St. Louis, USA, containing killed *M. tuberculosis*, was injected into the plantar surface of the hind left paw of 60 white outbred rats (females) under anesthesia.[16] The body weight range of the animals was 235 ± 18.17, aged 5 to 6 months. The experiment used an intact control (healthy rats; n = 30), the control group (CFA; n = 30), receiving intra-articular injection of saline 7 days after arthritis modeling, and the experimental group (CFA + AB; n = 30), receiving intra-articular administration of AB (5.00 mg in saline solution) 7 days after arthritis modeling. The AB was made from rat tendons and the dispersed form was used (particle size: 50.00 - 80.00 µm). The AB manufacturing technique was developed at the Federal State Budgetary Institution, All-Russian Center for Eye and Plastic Surgery, the Ministry of Health of the Russian Federation, Ufa, Russia. The biomaterial was manufactured in accordance with TU 42-2-537-87, certified and approved for use in clinical practice by order of the USSR Ministry of Health (No. 87 901-87 dated July 22, 1987). During the experiments, the international principles of the Helsinki Declaration on the Humane Treatment of Animals (2000) and the Federal Law of the Russian Federation on the Protection of Animals from Cruelty dated January 1, 1997 were followed. Thirty-seven days after the start of the experiment, the animals were removed from the experiment, 1.00 mL blood was taken from the tail vein of each animal for hematological analysis, and the knee joint with surrounding tissues was harvested for standard histological examination. Histological sections were stained with Hematoxylin and Eosin using the Van Gieson method, and studied and photographed using a light microscope (DMD108; Leica, Wetzlar, Germany). A complete blood test was performed on a SYSMEX XS 1000i hematology analyzer (Sysmex Corp., Kobe, Japan). All quantitative calculations were performed using the Statistica 10 Software package (Dell Inc., Austin, Texas).[17] Inter-group comparisons of blood parameters were carried out using the non-parametric Mann-Whitney test.[18] Assessment of inter-group differences in the mean value of cartilage thickness measurements of the knee joint of rats was carried out using the Fisher's one-way analysis of variance.[19]

The Ethics Committee of the Bashkir State Medical University, Ufa, Russia, approved the study. All manipulations were carried out in accordance with the provisions of the order of the Ministry of Health of the Russian Federation dated 01.04.2016 No. 199n, on Approval of the Rules of Good Laboratory Practice.

Results

The AA is characterized by severe progressive damage to the joints and internal organs of experimental animals. The administration of CFA into the rats' paws caused the development of an immune response, characterized by significant swelling of the paw and joints even at the end of the experiment. When conducting experimental work, the assessment of the edema degree is used as one of the diagnostic markers of the disease development.[7,8]

Edema was detected in the first days after the administration of CFA, in contrast to intact rats (Figs. 1A - 1C). By the end of the second week in the control group after administration of the adjuvant, the majority of rats (86.60%) began to show signs of arthritis in the form of severe swelling and deformation of the limbs, mainly in the area of the inter-phalangeal joints (Fig. 1D).

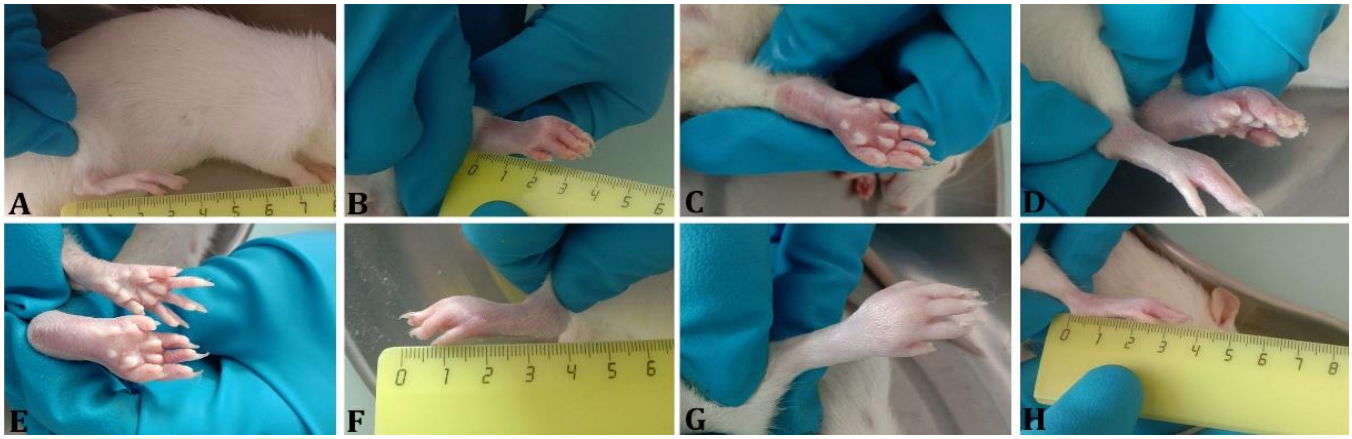

Fig. 1. Effect of dispersed allogenic biomaterial (AB) on the development of complete Freund's adjuvant (CFA) arthritis in mature female rats. **A)** Paw of an intact rat, **B)** Paw of a rat from the control group on the 2nd day after the administration of CFA, **C)** Paw of a rat from the experimental group with edema and erythema on the 2nd day after the use of CFA and before the administration of AB, **D)** Paw of a rat from the control group with edema and deformation at the end of the 2nd week after the administration of CFA, **E)** Paw of a rat from the control group (lower image) and paw of a rat from the experimental group (upper image) at the end of the experiment, **F)** Paw of a rat from the experimental group with signs of edema reduction 1 week after the administration of AB, **G)** Paw of a rat from the experimental group 2 weeks after the administration of AB, and **H)** Paw of a rat from the experimental group after the administration of AB at the end of the experiment.

After 2 - 3 weeks, in approximately 31.00% of animals, generalization of the pathological process was determined, affected the joints of the contralateral hind paw or joints at the base of the tail. Swelling of the animals' limbs persisted to varying degrees throughout the experiment (Fig. 1E). In 14.40% of the animals in the control group, a gradual decrease in the severity of edema and erythema was observed throughout the experiment, indicating the absence of arthritis development in these rats. During the first week after the administration of CFA, the animals experienced a slight slowdown in the dynamics of weight gain, which may also reflect the consequences of the development of an acute inflammatory process. Starting from the second week, the dynamics of rats' weight gain was stabilized, differing little from that of intact rats and animals of the experimental group.

By the end of the second week, in rats of the experimental group, received intra-articular injection of AB 7 days after the use of CFA, the clinical signs of disease development noticeably stopped in the form of a decrease in swelling and redness of the paw and peri-articular areas (Fig. 1F). After using the biomaterial, a gradual weakening of the clinical signs was observed in all animals, which may indicate a decrease in the development of arthritis in these rats. This is probably why we did not observe any noticeable signs of generalization of the pathological process affecting the joints in the experimental group (Fig. 1G). Clinical symptoms of arthritis in the experimental group of rats completely disappeared by the end of the experiment (Fig. 1H). The dynamics of weight gain in animals, even if it decreased during the first week before using AB, differed little from that of intact animals in the future.

Analysis of the blood test results showed that the variation of most parameters (especially in the control group) cannot be correctly considered as a modification of the normal distribution. Thus, inter-group comparisons were carried out using the non-parametric Mann-Whitney test, and the median and lower and upper quartiles were used to describe the results. In the experimental group after the administration of AB, as well as intact group of rats, the level of leukocytes in the blood was approximately twice and significantly (6.20×10^9 L^{-1}; $p \leq 0.001$) lower than rats of the control group (11.20×10^9 L^{-1}). The level of segmented neutrophils in the experimental group was significantly (39.00%; $p \leq 0.001$) lower than control group (75.00%), as well as band neutrophils. The level of lymphocytes in the intact and experimental groups turned out to be significantly (39.00%; $p \leq 0.001$) higher than control group (12.50%; Table 1).

The level of monocytes in the blood of rats in the intact (7.00%) and control (2.20%) groups was significantly ($p < 0.02$) lower than experimental group (13.00%). The level of eosinophils in the experimental group was lower than control group, but not significantly ($p = 0.13$), due to high intra-group variation in the intact group. The number of erythrocytes in the blood of the intact and experimental groups was significantly higher (4.77×10^{12} L^{-1}; p = 0.0001) than control group (3.77×10^{12} g L^{-1}; $p = 0.0002$). The same was happened regarding to the hemoglobin content. In the control group, the hemoglobin content was significantly lower (120 g L^{-1}; $p \leq 0.001$) than intact (134 g L^{-1}) and experimental (135 g L^{-1}) groups. With all of the above, changes in the parameters of white and red blood cells against the background of a pronounced inflammatory process after the administration of CFA were marked by an increase in the total number of leukocytes.

In the majority of rats in the control group, histological preparations revealed dystrophic and destructive changes in the articular apparatus, manifested in varying degrees of severity. In the synovial membrane, there were signs of pronounced proliferation of synoviocytes, tissue hyperplasia, swelling, and cellular infiltrates containing lymphocytes, plasma cells, macrophages, and fibroblasts. The synovial membrane underwent hyperplasia and formed the so-called pannus, penetrated quite deeply into the articular cavity. In the perichondrium, in places there was disorganization of the connective tissue, expressed in thinning and disintegration of the fibrous layer, up to the complete destruction of the surface layer. The thickness of the superficial and cambial layers in the preserved cartilage decreased.

In the animals of the experimental group, a month after intra-articular administration of AB, the morphological picture was different. In the majority of rats, pronounced signs of tissue hyperplasia, swelling, and inflammatory cell infiltrates characteristic of arthritis were absent in the synovial membrane, and pannus was not detected. The structure of the synovial membrane was close to normal, although some increased folding was detected (Fig. 2A).

Table 1. Blood parameters of rats in different groups. Data are presented as median (lower quartile - upper quartile).

Parameters	Intact group	Control group	Experimental group
Leukocytes ($\times 10^9$ g L^{-1})	6.20 (5.10 - 6.90)	11.70 (10.30 - 14.20)	6.20 (5.70 - 6.80)
Band neutrophils (%)	1.00 (0.00 - 1.00)	6.00 (5.00 - 7.00)	1.00 (1.00 - 2.00)
Segmented neutrophils (%)	49.00 (48.00 - 57.00)	75.00 (70.00 - 78.00)	39.00 (37.00 - 43.00)
Lymphocytes (%)	39.00 (35.00 - 41.00)	12.50 (11.00 - 15.00)	40.00 (39.00 - 42.00)
Monocytes (%)	7.00 (5.00 - 9.00)	3.00 (2.00 - 4.00)	13.00 (11.00 - 15.00)
Eosinophils (%)	3.00 (0.00 - 6.00)	1.00 (1.00 - 2.00)	2.50 (1.00 - 3.00)
Erythrocytes ($\times 10^{12}$ L^{-1})	5.00 (4.50 - 6.10)	3.70 (3.30 - 4.60)	4.60 (4.10 - 6.50)
Hemoglobin (g L^{-1})	134 (130 - 137)	120 (116- 124)	135 (129 - 140)

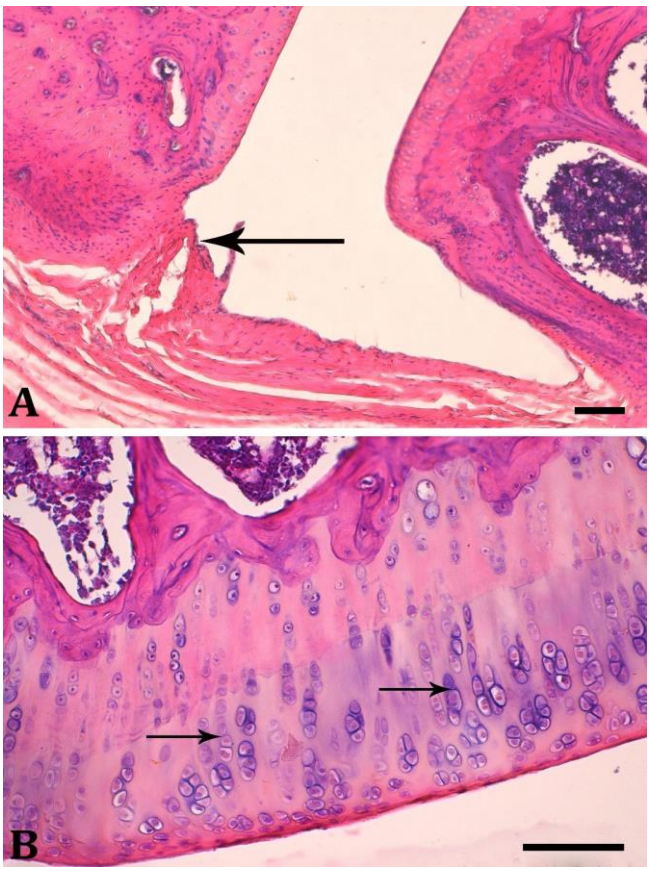

Fig. 2. The structure of the knee joint of rats in the experimental group on the 37th day of the experiment. **A)** The synovial membrane is indicated by an arrow, and **B)** Structure of articular cartilage. Isogenic groups of chondrocytes are evident (arrows), (Hematoxylin and Eosin Staining; Bars = 100 µm).

The synoviocytes in the surface layer of the membrane were located in one to two rows and no signs of pronounced cell proliferation were observed. The articular cartilage also did not show any signs of pathological changes (Fig. 2B). There were no signs of fiber disintegration, thinning, or destruction of the perichondrium. Chondrocytes in articular cartilage had a round shape, formed isogenic groups, and were found in vertical rows.

In three rats of the experimental group, histological preparations of joints revealed relatively weak signs of the development of AA in the form of locally increased proliferation of synovial cells, weak peri-vascular infiltration of tissues by cellular elements, and congestion in individual vessels with slight swelling of their walls, but there were no pronounced pathological changes in the structure of cartilage in the knee joint, if we do not take into account some of its thinning. In some rats of the experimental group, in comparison with intact animals, the thickness of the articular cartilage decreased slightly. Therefore, the thickness of the cartilage layer was measured in rats of all studied groups.

The analysis showed that the average level of articular cartilage thickness in rats strictly depended on their group membership ($\eta^2 = 0.89$, $F = 610$, and $p \leq 0.0001$). As can be seen in Figure 3, the highest average cartilage thickness (194.30 ± 11.90 µm) occurred in the intact group. In the experimental group, 30 days after AB administration, the average thickness of the articular cartilage of rats turned out to be quite close to that of the intact group (169.80 ± 19.60 µm), but still significantly ($p < 0.0001$) lower. The smallest cartilage thickness occurred in the control group (88.10 ± 15.20 µm).

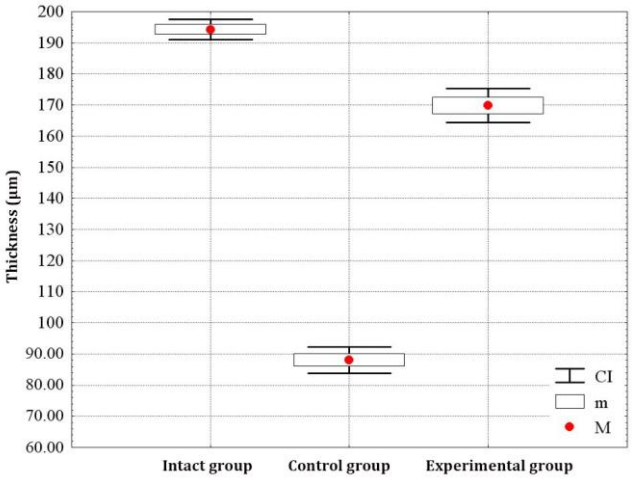

Fig. 3. Average thickness (µm) of hyaline cartilage of the knee joint of rats in three groups. The horizontal axis shows the groups. The vertical axis shows the thickness of articular cartilage. CI: Confidence interval; m: Standard error of the mean; M: Arithmetic mean.

Discussion

Allogeneic biomaterials for joint pathologies are a direction in veterinary orthopedics and rehabilitation veterinary medicine, conditioned by extensive research.[5] Over the past decades, the accumulated experience in the use of allogeneic tissues has opened up new perspectives in the treatment of joint pathologies, such as osteoarthritis and traumatic injuries.[6,7] These donor-derived materials show high biocompatibility and are able to facilitate the restoration of damaged tissues by simulating the natural environment. [5-8]

A retrospective of the use of ABs demonstrates not only their effectiveness but also the variety of methods of use.[7-11] For example, the introduction of alloplants in surgical practice made it possible to significantly speed up the healing process, improve joint function, and reduce the risk of complications.[9-11] Particular attention is also paid to the immune response and potential changes in blood composition, stimulating further research.[7,8]

Thus, intra-articular administration of AB to experimental rats while using CFA neutralized the manifestation of signs of a generalized inflammatory

process in the joints and reduced the degree of destructive changes in the articular apparatus, preserving the structure of the cartilaginous layer compared to the AA animal models pathology. Along with this, the use of AB allowed to stabilize most of the red and white blood cells parameters of experimental rats, as well as significantly raise the reduced level of monocytes, the precursors of macrophages. It is known that macrophages localized at the site of AB implantation are producers of several cellular growth factors and cytokines, including metallo-proteinases, promoting proteolytic degradation of the pathologically altered inter-cellular matrix.[9,10] Macro-phages, being representatives of the reticuloendothelial system of the body, actively participate in the protection of tissues not only from foreign antigens but also, probably, from auto-antigens. They phagocytize cell-tissue detritus, stimulate and regulate the immune response, induce an inflammatory response, and participate in reparative processes and the exchange of components of the extra-cellular matrix, including the constituent substances of AB introduced into the joint.[11] Therefore, the weakening of the degree of the inflammatory process after the use of AB, manifested morphologically in the absence of hyperplasia of the synovial membrane in the form of pannus and a decrease in the number of lymphocytes, and the preservation of the basic structure of the cartilage and bone tissue of the knee joint can be due to both the biochemical composition of the AB and a change in the spectrum of cytokines determined by the cellular micro-environment after the introduction of the biomaterial.

It has been established that in addition to collagen, AB made from connective tissue contains a significant amount of glycosaminoglycans (13.80%) in the form of hyaluronic acid, chondroitin sulfate, and heparan sulfate.[20] They are the ones that can have a chondro-protective effect. glycosaminoglycans, especially hyaluronic acid, are an integral part of synovial fluid and are necessary for the formation of articular cartilage proteoglycans.[2] With the development of arthritis and arthrosis, pathologically altered chondrocytes contribute to the production of defective basic substance of cartilage tissue; the tissue begins to undergo depolymerization and the content of its glycosaminoglycans decreases, especially the amount of hyaluronic acid.[20] The content of the latter determines the lubricating properties of the synovial fluid, being squeezed out of the cartilage matrix when mechanical loads on the joint increase.

Intra-articular administration of AB during AA modeling exhibited osteo- and chondro-protective effects, providing positive anti-inflammatory and symptom-modifying effects and weakening the manifestation of pathomorphological changes in joints in experimental rats. The use of AB along with CFA made it possible to stabilize most of the red and white blood cells parameters of experimental rats.

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Conflict of interest

The authors declare no obvious or potential conflicts of interest related to the publication of this article.

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