MORPHOLOGICAL CHANGES IN TISSUES OF THE KNEE JOINT OF THE RABBIT BECAUSE OF EXPERIMENTAL OSTEOARTHRITIS

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Abstract. *Osteoarthritis is the most common disease of the joints, in which there is a disequilibrium between the processes of recovery and destruction in the cartilage and bone located under the cartilage, as well as in surrounding tissues: articular capsules, ligaments, muscles. For osteoarthritis, the structure of articular cartilage changes due to the loss of proteoglycans, which leads to its destruction, with atrophy of chondrocytes and the formation of new bone tissue around the affected joint. That is why studying morphological changes in the tissues of the knee joint allows us to confirm the presence of osteoarthritis. Studies have shown that chronic retinol acetate can be used to simulate chronic sclerosing osteoarthritis. Damage caused by retinol acetate can be identified as chronic sclerosing osteoarthritis. In this case, there is a thinning of the hyaline cartilage or its complete disappearance, the growth of dense fibrous connective tissue. Histologic studies have shown that there are horizontal unbranched cracks (fissures) in the cartilage tissue and in the fibrous connective tissue, the germs are thinned or absent. The main signs of such a defect are almost fully expressed at 7 days after the injury and classified as a 5-degree defeat (according to the classification of OARSI, 2000).*

Keywords: knee joint, ostearthrosis modeling, rabbits, retinol acetate, chronic sclerosing osteoarthritis

UDK 576.6–57.085.23

THE INFLUENCE OF ALLOGENEIC BONE MARROW DERIVED MESENCHYMAL STEM CELLS ON INDICATORS OF FUNCTIONAL STATE OF IMMUNE ORGANS IN MICE C57BL/6

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Abstract. *The studies were conducted on 2-3-months-old males of mice weighing 20-24 g. Our work was to study the functional state of the organs of the immune system of C57Bl/6 mice after introduction of allogeneic*

MSCs of bone marrow origin. Obtaining and cultivating of MSCs were carried out in a sterile laminar box with compliance of conditions of asepsis and antiseptics. C57Bl/6 mice bone marrow aspirate cultured in a CO2 incubator at 37 oC and 5 % CO2 in DMEM with 10-15 % of fetal bovine serum, 1 % of antibiotic-antimycotic solution (Sigma-Aldrich, USA). The following groups of animals were formed: 1 group – intact (control group); 2 group - animals, to whom 0.5 ml of 0.9 % NaCl solution (placebo) were injected into the caudal vein; 3 group – animals, to whom 104 of allogeneic MSCs in 0.5 ml of phosphate buffer solution were injected into the caudal vein. The weight index, cellularity of thymus and spleen in C57Bl/6 mice investigated after the introduction of MSCs. The administration of allogenic mesenchymal stem cells of the bone marrow origin affects on the central and peripheral organs of the immune system. Administration of allogenic mesenchymal stem cells cause a significant increase in the content of lymphoid cells in the thymus at 7 and 18 days by 72 and 39 %, respectively ($p < 0.01$, $p < 0.05$) compared to the control. Administration of allogenic mesenchymal stem cells cause a significant increase in the weight index of the spleen and its cellularity at the 7 and 18 days of the immune response by 26 and 17 %, respectively ($p < 0.01$, $p <0.05$) compared to the control. the weight index of the spleen and it cellularity a significant increase at the 7 and 18 days of the immune response by 26 and 17 %, respectively ($p < 0.05$) compared to the control after administration of allogenic mesenchymal stem cells.

Keywords: mice, weight index, cellularity, thymus, spleen, allogenic mesenchymal stem cells

Introduction. The first link of the systemic response of the body to the biologically material of foreign origin is immune system. Thymus, as one of the central organs of the immune system, where maturation and differentiation of T-lymphocytes occurs, provides cellular adaptive immune response, as well as participation in the regulation of humoral immune responses via production of cytokines. The spleen, as the peripheral organ of the immune system, contains T- and B-dependent regions and provides antigen-dependent proliferation and differentiation of T- and B-lymphocytes, their activation, as well as secretion of antibodies, elimination of old red blood cells and foreign particles. The functional state of the thymus and spleen depends on its structural organization, and the polymorphism of the cellular elements [1].

Analysis of recent researches and publications. Among the current scientific data, there are numerous studies about the successful use of MSCs for therapeutic purposes [2, 6], but the mechanism of action and functioning of the immune system of recipients to the injected MSCs has not yet been fully understood. Possible variants of action of MSC are induction of a local immunosuppressive or immunomodulatory effects on the immune cells and a low immunogenicity of MSCs [7, 9]. Low immunogenicity of MSC is one of the characteristic features. Allogeneic MSC does not cause a rejection reaction. MSCs store viability and proliferative activity in a site of injection, even without immunosuppression [5, 11]. This is due to the minor expression of molecules

of the major histocompatibility complex of the first class that provide antigen presentation to killers destroying foreign agents or stimulating other cells (B-lymphocytes and macrophages) [18]. Perhaps, these mechanisms are combined and interweave with each other, including direct contact of MSCs with immune cells [14]. Also MSCs influences through the production and release of soluble factors - cytokines, prostaglandins [10, 17].

The inserted mesenchymal stem cells causes restoring not only of pathologically altered organs or tissues, but also affects on the whole organism. A number of studies are devoted to the impact of MSCs on manifestations of experimental diseases with autoimmune processes [2, 9]. There are a number of studies that emphasize that use of MSCs in different phases of the course of the pathological process causes a different effects [3, 13]. Thus, the use of allogeneic or xenogeneic MSCs can reduce the intensity of autoimmune encephalomyelitis in mice, if the MSCs are administered at the peak of the disease, but not during its stabilization.

As to the effect of MSC on immune cells, there are also opposite data [8, 19]. For example, a number of authors have confirmed the blocking effect of MSCs on B cells, while other researchers have revealed increased B-lymphocyte proliferation, their differentiation into plasma cells and increased IgG production [4, 19].

Thus, due to contradictory of the data of the impact of MSCs on the organs of immune system and the limited number of literature reports regarding to the functional status of immune organs, in particular thymus and spleen, after introduction of MSCs, these issues require further study.

Purpose. The purpose of our work was to study the functional state of the organs of the immune system of C57Bl/6 mice after introduction of allogeneic MSCs of bone marrow origin.

Methods. The studies were conducted on 2-3-months-old males of C57BL / 6 mice weighing 20-24 g. All studies were conducted in accordance with the Rules of Good Laboratory Practice and Use of Experimental Animals and in accordance to Compliance with the Law of Ukraine "On the Protection of Animals from Cruel Treatment" and the "International European Convention on the Protection of Animals Used for Experimental and Other Scientific Purposes ".

MSCs obtaining from bone marrow of mice. Obtaining and cultivating of MSCs were carried out in a sterile laminar box with compliance of conditions of asepsis and antiseptics. The mice were euthanized, their femur, tibia and shoulder bones were removed, and washed three times with sterile phosphate buffer solution with the addition of 1 % antibiotic-antimycotic solution (Sigma-Aldrich, USA). Bone marrow was washed out from the diaphyses of removed bones by using the Dulbecco's Modified Eagle's Medium (DMEM). Bone marrow aspirate was added to culture dishes filled with DMEM, 10-15 % of fetal bovine serum, 1 % of antibiotic-antimycotic solution (Sigma-Aldrich, USA) and cultured in a CO2 incubator at 37 oC and 5 % CO2. The culture medium was partially or completely changed by fresh medium every 3 days during cultivation. After formation of cells monolayer at 80-90 %, cells were removed with trypsin-ethylenediaminetetraacetic acid solution (EDTA), washed with phosphate buffer and placed in Petri dishes for

cultivation [16]. Passaging the cells provided a reduction of heterogeneity of cell culture and the development of biological material for transplantation. For transplantation were used MSCs of the 4 passage.

MSCs administration to mice. The following groups of animals were formed: 1 group – intact (control group); 2 group – animals, to whom 0.5 ml of 0.9 % NaCl solution (placebo) were injected into the caudal vein; 3 group – animals, to whom 104 of allogenic MSCs in 0.5 ml of phosphate buffer solution were injected into the caudal vein.

Estimation of weight index of thymus and spleen of mice after introduction of MSCs. Indicators of the weight of peripheral lymphoid organs relative to the body weight (weight index) of animals were evaluated at 7, 18 and 25 days after the introduction of MSCs. The mice were pre-weighed for weight control. At each study period in each group, 3 animals were euthanized and the weight index of lymphoid organs and their cellularity were studied.

Euthanasia of animals was carried out with using of carbon dioxide, lymphoid organs - thymus and spleen - were removed and determined its mass. Indices of lymphoid organs in relation to the weight of the animal were calculated according to the formula:
Weight index (%) = weight of the lymphoid organ / weight of the animal x 100.

Evaluation of cellularity of the thymus and spleen after the introduction of MSCs. To assess the content of lymphocytes in lymphoid organs, the latter were weighed. Whole thymus and 50 mg of spleen were triturated and filtered through the kapron tissue. After that, the cell homogenate was applied to the gradient of ficoll-urografin (density 1.077) in a ratio of 3:2. The test tubes were centrifuged at a rate of 1500 rpm for 30-40 minutes. After centrifugation the layer of lymphocytes which was above the gradient was collected by a Pasteur pipette and washed twice with an arbitrary amount of Hanks' solution by centrifugation at a rate of 1500 for 10 minutes. After washing 1 ml of Hanks's solution was added to lymphocytes. Lymphocytes were counted in the chamber Goryaev. Calculation of the cells of lymphoid organs was performed on 1 mg of tissue.

Results. Immune homeostasis depends on the ratio of proliferation and apoptosis of thymocytes [12, 20] which have not been practically investigated under the use of MSCs.

Increased of mitotic activity of thymocytes under normal conditions is due to antigenic stimulation. In this case, as antigenic stimulus were introduced MSCs. Despite numerous publications which disclose the immunological properties of these cells and confirme that they have immunosuppressive effects, some data research results demonstrate that MSCs under certain conditions can be eliminated by cells of the immune system of the recipient animal, because they carry signs of foreignness [10].

Increasing cellularity of thymus after administration of MSCs can be due to insufficient number of administrated cells for realization of their immunosuppressive manifestations [15] and low concentration of immunosuppression factors synthesized by MSCs.

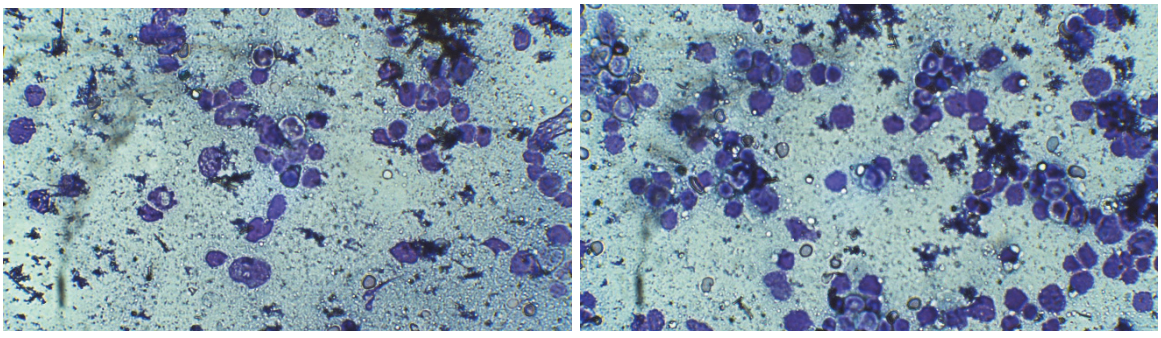
The results of the studies indicate that administered MSCs have a stimulating effect on the proliferative activity of thymocytes in vivo, as indicated by an increase in thymic cellularity at 7 and 18 days of study compared with control and placebo at 79 % and 32 % respectively (Table 1).

1. Cellularity and the weight index of thymus of C57Bl / 6 mice after administration of allogeneic MSCs, M + m, n = 9, x106/mg, %

Groups of an animals / Terms of study	Intact (*n* = 6) ($x10^6$ / mg)	Administration of 0.89 % NaCl, placebo (*n* = 9) ($x10^6$ / mg)	Administration of MSCs (*n* = 9) ($x10^6$/мг)	Weight index of thymus after administration of MSC,%
7 day	1,4 ± 0,1	1,9 ± 0,2	2,5 ± 0,3** ^{v}	0,21 ± 0,03* ^{v}
18 day	1,4 ± 0,1	1,3 ± 0,1	2,9 ± 0,1*** ^{vvv}	0,20 ± 0,1* ^{v}
25 day	1,7 ± 0,1	1,4 ± 0,1	2,5 ± 0,2* ^{vv}	0,18 ± 0,1* ^{v}

*– p < 0,05, **– p ≤ 0,01, ***– p ≤ 0,001, compared to a group of intact animals; ^{v} –p <0,05, ^{vv}– p ≤ 0,01, ^{vvv}– p ≤ 0,001, compared to placebo group

Under the influence of MSCs, a significant increase in the content of lymphoid cells in the thymus lasts until the 18th day of the study. The thymus contains T-lymphoblasts, ripening and mature lymphocytes, supporting and secretory cells of the thymus stromal component (Fig. 2).



a) *b)*

Fig. 2. Lymphoid cells of thymus on the 7 day of the study: a) – intact group, b) – after the introduction of MSC, x 100

On the 25 day after the introduction of MSC, the content of lymphoid cells in the thymus was reduced compared to 18 day of study, but was significantly higher than in intact animals and after administration of 0,89 % NaCl. This indicates a gradual decrease in the proliferative activity of the thymocytes and the involution of the organ.

A reliable (p < 0.05) positive correlation of thymus cellularity with weight index was established after administration of MSCs.

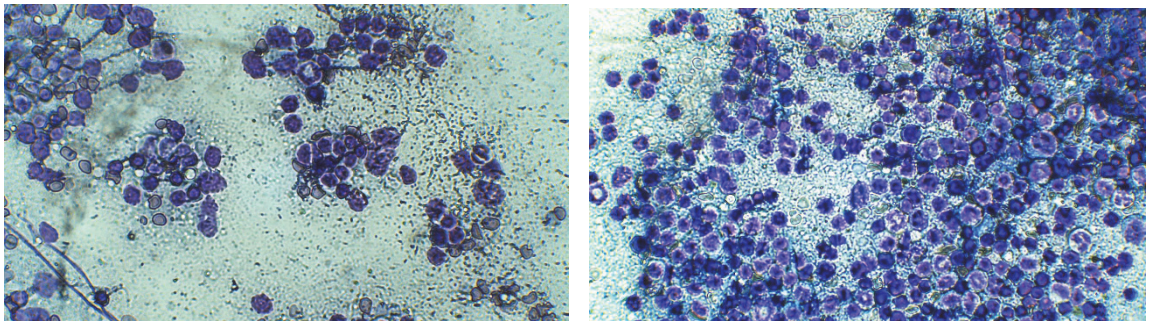
The spleen, as the peripheral organ of the immune system, is also involved in the process of forming an immune response to the antigen. After the administration of MSC, the content of lymphoid cells in the spleen significantly exceeded the parameters of cellularity of spleen of intact animals (Table 2).

2. Cellularity and the weight index of spleen of C57Bl/6 mice after administration of allogeneic MSCs, M + m, n = 9, x10^6 / mg, %

Groups of an animals / Terms of study	Intact (*n* = 6) (x10^6 / mg)	Administration of 0.89% NaCl, placebo (*n* = 9) (x10^6 / mg)	Administration of MSCs (*n* = 9) (x10^6/мг)	Weight index of thymus after administration of MSC, %
7 day	2,7 ± 0,1	2,9 ± 0,1	3,4 ± 0,1** ^{v}	0,65 ± 0,02*
18 day	2,7 ± 0,1	2,8 ± 0,4	3,1 ± 0,1* ^{v}	0,62 ± 0,02*
24 day	2,7 ± 0,1	2,5 ± 0,1	1,9 ± 0,2* ^{v}	0,42 ± 0,01*

*– p ≤ 0,05, ** – p ≤ 0,01, compared to a group of intact animals; v – p ≤ 0,05, compared to placebo group

On the 7th day after administration of MSCs, cellularity of spleen was significantly higher by 26 % (Figure 3) than in control animals and by 17% compared to animals which were administered 0.89 % NaCl. Such changes indicate a direct response of the spleen to the introduction of stem cells. The weight index of the spleen directly correlates with the content of the lymphoid cells in it.



a) *b)*

Fig. 2. Lymphoid cells of spleen on the 7th day of the study: A – intact group, B – after the introduction of MSC, x 100

On the 18th day after administration of MSCs spleen lymphoid cell content was also significantly higher than that of the other two groups, but was less than at the 7th day.

On the 24th day of the study the number of lymphoid cells in the spleen was significantly reduced by 42 % compared to the control animals and by 32 % compared to the animals which were administered 0.89 % NaCl. The content of lymphoid cells in spleen on the 24 day after stem cells administration correlates with the weight index of spleen, indicating its involution.

Discussion. The administration of allogenic mesenchymal stem cells of the bone marrow origin affects on the central and peripheral organs of the immune system.

Administration of allogenic mesenchymal stem cells cause a significant increase in the content of lymphoid cells in the thymus at 7 and 18 days after the administration of stem cells by 72 and 39 %, respectively ($p < 0.01$, $p < 0.05$) compared to the control.

Administration of allogenic mesenchymal stem cells cause a significant increase in the weight index of the spleen and its cellularity at the 7 and 18 days of the immune response by 26 and 17 %, respectively ($p < 0.01$, $p < 0.05$) compared to the control.

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ВПЛИВ АЛОГЕННИХ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН НА ПОКАЗНИКИ ФУНКЦІОНАЛЬНОГО СТАНУ ІМУННИХ ОРГАНІВ МИШЕЙ C57BL/6

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Анотація. *Дослідження проводили на самцях мишей С57BL /6 вагою 20-24 г віком 2-3 місяці. Маніпуляції з отримання первинного матеріалу та культивування МСК здійснювали в стерильному боксі з дотриманням усіх правил асептики й антисептики. Аспірат кісткового мозку мишей С57BL /6 культивували за температури 37 °С, 5 % CO_2, 95 % вологості у CO_2 інкубаторі у середовищі DMEM, з додаванням 10-15 % фетальної сироватки бичків, 1 % антибіотика-антимікотика (Sigma-Aldrich, USA).*

Для проведення досліджень було сформовано наступні групи тварин: 1 група – інтактні (контрольна група); 2 група – інтактні тварини, яким у хвостову вену вводили 0,5 мл 0,9 % розчину NaCl (плацебо); 3 група – тварини, яким у хвостову вену вводили 10^4 алогенних МСК з кісткового мозку в 0,5 мл фосфатно-буферного розчину. Досліджували ваговий індекс, вміст лімфоїдних клітин тимусу та селезінки мишей С57Bl/6 за введення МСК з кісткового мозку. Трансплантація алогенних мезенхімальних стовбурових клітин з кісткового мозку чинить вплив на центральні і периферичні органи імунної системи. За впливу алогенних МСК з кісткового мозку відбувається достовірне підвищення вмісту лімфоїдних клітин тимусу на ранніх і пізніх етапах імунної відповіді на 72 і 39 % відповідно ($p \leq 0,01$, $p \leq 0,05$). За впливу алогенних МСК з кісткового мозку реєструється достовірне підвищення вагового індексу селезінки та її клітинність на 7 та 18 добу імунної відповіді на 26 і 17 % відповідно ($p \leq 0,01$, $p \leq 0,05$). На 24 добу в селезінці реєструються процеси інволюції із достовірним зниженням показника вмісту лімфоїдних клітин у порівнянні з інтактними тваринами та групою плацебо на 42 і 32 % відповідно ($p \leq 0,05$).

Ключові слова: миші, алогенні стовбурові клітини, кістковий мозок, ваговий індекс, тимус, селезінка

ВЛИЯНИЕ АЛОГЕННЫХ МЕЗЕНХИМАЛЬНЫХ СТВОЛОВЫХ КЛЕТОК ИЗ КОСТНОГО МОЗГА НА ПОКАЗАТЕЛИ ФУНКЦИОНАЛЬНОГО СОСТОЯНИЯ ИММУННЫХ ОРГАНОВ МЫШЕЙ С57BL/6

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Аннотация. *Исследования проводили на самцах мышей С57BL/6 весом 20-24 г в возрасте 2-3 месяца. Манипуляции с первичным материалом для культивирования МСК осуществляли в стерильном боксе с соблюдением всех правил асептики и антисептики. Аспират костного мозга мышей С57BL/6 культивировали в CO_2 инкубаторе при температуре 37 ° С, 5 % CO_2, 95 % влажности в среде DMEM с добавлением 10-15 % фетальной сыворотки бычков, 1 % антибиотика-антимикотика (Sigma-Aldrich, USA). Для проведения исследований было сформировано следующие группы животных: 1 группа – интактные (контрольная группа) 2 группа – животные, которым в хвостовую вену*