ПРИМЕНЕНИЕ СТВОЛОВЫХ КЛЕТОК В НАПРАВЛЕННОЙ РЕГЕНЕРАЦИИ КОСТНОЙ ТКАНИ

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Резюме. Современный уровень медицины позволяет все больше изучать и разрабатывать материалы и методики восстановительного лечения, которые бы опирались на иммунологические механизмы костной репарации. Одним из перспективных направлений в направленной костной регенерации является применение мезенхимальных стволовых клеток. Интерес в применении МСК связан с их способностью регулировать воспалительный процесс, и участвовать в формировании новых костных структур, тем самым обеспечивая воспроизведение процессов естественной репарации. Эффекторное влияние МСК на воспалительный процесс обусловлен, прежде всего, их способностью формировать специфическое микроокружение. Низкая экспрессия MHC-II и CD80/CD86 определяет их низкую иммуноконфликтность, продукция PGE2 и NO обеспечивает иммуносупрессию в месте заселения МСК, а продукция TGF- 1, IDO и IL-10 оказывает иммуномоделирующее действие. Более того, особое внимание к себе привлекает способность этих клеток дифференцироваться в остеогенный фенотип. Данный сложный многостадийный процесс сопровождается выделением ряда биологически активных веществ, влияющих на костную репарацию. Синтез ALP, BSP и в последующем Gla-protein и OPN обуславливают синтез внеклеточного матрикса и его последующую минерализацию. Регуляция данного процесса обеспечена действием Runx2, который активирует дифференцировку МСК по остеогенному пути. Данные эффекты МСК были взяты за основу в процессе разработки новой методики лечения атрофий костной ткани. Для выполнения поставленных задач была проведена разработка модели атрофии костной ткани, выполнена разработка препарата, содержащего в своем составе МСК, а также проведено экспериментальное исследование для оценки эффективности разработанной методики. В качестве основных критериев оценки качества проведенного лечения были взяты данные клинического и лабораторного исследований. Учитывались визуальные изменения исследуемого участка, по сравнению с аналогичным участком в разработанной модели атрофии, оценивались

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Образец цитирования:
Н.С. Чумаков, К.А. Хлыстова, Н.Г. Саркисян, Ф.А. Фадеев, М.М. Мамедов «Применение стволовых клеток в направленной регенерации костной ткани» // Медицинская иммунология, 2023. Т. 25, № 3. С. 691-696.
doi: 10.15789/1563-0625-AOS-2724

For citation:
N.S. Chumakov, K.A. Khlystova, N.G. Sarkisyan, F.A. Fadeev, M.M. Mamedov "Application of stem cells in guided bone regeneration", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2023, Vol. 25, no. 3, pp. 691-696.
doi: 10.15789/1563-0625-AOS-2724

DOI: 10.15789/1563-0625-AOS-2724

параметры ОАК, отражающие интенсивность протекания воспалительной реакции. Выполненное экспериментальное исследование позволяет определить разработанную методику лечения как способную в полной мере воссоздать условия процессов костной репарации, с учетом оптимизации иммунных реакций организма и процессов репарации, без дополнительного влияния извне, получить предсказуемые и контролируемые результаты. Имеющиеся данные исследования позволяют определить эффективность разработанной модели и методики лечения, а также дальнейший вектор проведения исследований.

Ключевые слова: стволовые клетки, атрофия, репарация, костная регенерация, костный дефект

APPLICATION OF STEM CELLS IN GUIDED BONE REGENERATION

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Abstract. Modern medicine allows us to study and develop materials and methods of restorative treatment that would be based on the immunological mechanisms of bone repair. One of the promising directions in guided bone regeneration is the use of mesenchymal stem cells. Interest in MSCs is associated with their ability to regulate the inflammatory process, and directly participate in the formation of new bone structures, thereby providing a physiological repair process. The effector impact of MSCs on the inflammatory process due to their ability to form a specific microenvironment. Low expression of MHC-II and CD80/CD86, the production of PGE2 and NO determines their low immunoconflict, and the production of TGF-β1, IDO and IL-10 has an immunomodulating effect. The ability of MSCs to differentiate into an osteogenic phenotype is accompanied with the synthesis of ALP, BSP and, subsequently, Gla-protein and OPN determine the synthesis of the extracellular matrix and its subsequent mineralization. This process is provided by the action of Runx2, which activates the differentiation of MSCs along the osteogenic pathway. These effects of MSCs were taken as the basis for the development of a new method for the treatment of bone atrophy. To accomplish the task set, a model of bone tissue atrophy and a drug containing MSCs was developed, and an experimental study was conducted to evaluate the effectiveness of the developed methodology. As the main criteria, data from clinical and laboratory studies were taken. Visual changes in the studied area were taken into account, compared with a similar area in the developed model of atrophy, the parameters of the complete blood count (CBC) were evaluated. The performed study allows us to determine the developed treatment method as capable of fully recreating the conditions of bone repair processes, taking into account the optimization of the body's immune reactions and repair processes, without additional external influence, to obtain predictable and controllable results.

Keywords: stem cells, atrophy, repair, bone regeneration, bone defect

Introduction

One of the most difficult issues of restorative dentistry we are facing these days is atrophy of the alveolar processes of the jaws [4]. Particular attention is drawn to the immunological mechanisms of the development of this pathology, as well as the possibility of optimizing the immunological aspects of bone repair [6]. The significance of these issues is due to complications that are associated with the development of bone loss, including a significant difficulty in restorative orthopedic and implant treatment, and the need for complex preparatory operations with a greater progression of pathology.

The development of modern medicine and the medical industry offers a variety of materials and techniques for guided bone tissue regeneration. The use of autogenous and artificial materials has good long-term results, but they are not able to fully

compensate for the processes of natural bone tissue regeneration [10]. This promotes the development of new treatment methods that have an effect on all parts of bone reparation.

One of such directions is cellular technologies. In recent years, the possibility of using mesenchymal stem cells (MSCs) has been actively studied. Interest in these cells is due to their ability to differentiate into cells of bone metabolism, as well as to produce a number of biologically active substances, among which growth factors, cytokines and various mediators are determined, thereby creating a microenvironment that determines the course of bone repair processes [8]. All this brings us as close as possible to the conditions of physiological regeneration with the formation of our own bone tissue.

Literature describes many examples of the successful use of MSCs in the restorative treatment of degenerative diseases of bone tissue [3, 9]. Methods for the treatment of articular cartilage necrosis, correction of post-traumatic facial deformities, treatment of bisphosphonate-associated osteonecrosis of the jaw bones is mentioned. In the treatment of bisphosphonate-associated osteonecrosis of the jaw, isolated and cultured culture of MSCs is preferred [5]. The effectiveness of treatment with this method is proved by a large number of publications in domestic and foreign literature. Many authors report that clinical success in the treatment of bisphosphonate-associated osteonecrosis of the jaw with MSCs has been observed even in patients receiving concomitant immunosuppressive therapy.

However, there is little data in the literature on the possibility of using MSCs for guided regeneration of the alveolar processes of the jaws. The low development of this topic dictates the need to pay more attention to the issues of stimulating endogenous regeneration, as well as the possibility of conducting direct regulation of this process. Thus, the issues of prevention and correction of bone tissue atrophy, in particular the atrophy of the alveolar processes of the jaws, remain promising areas and require the search for modern methods for correcting these conditions.

Materials and methods

The study was conducted at the Federal State Budget Institution of Science "Institute of Immunology and Physiology" of the Ural Branch of the Russian Academy of Sciences, and included several successive stages:

1. Development and implementation of a model of bone tissue atrophy;
2. Cultivation of MSCs (conducted at the State Autonomous Healthcare Institution of the Sverdlovsk Region "Institute of Medical Cellular Technologies");
3. Conducting an experimental study.

Before developing the model, the main criteria were formulated, the observance of which would make it possible to assess its effectiveness:

– Maximum proximity to the real conditions for the development of atrophy;
– Formation of a sufficient amount of atrophy for an adequate assessment of the expected and obtained results;
– Non-development of adverse reactions, prevention of distortion of long-term results;
– No need for external influence and ease of implementation.

At its core, the model of bone tissue atrophy is similar to traumatic tooth extraction (an extraction of a tooth, in which an intentional and accidental additional bone defect is formed, in the form of a break of the interradicular septum / cortical plate in the area of the socket of the extracted tooth), and does not require additional manipulations in the postoperative period. The process of reproducing the model consists of the following steps:

1. Under general anesthesia (Diethyl ether), an incision is made in the mucous membrane, from the lower incisor, along the most protruding part of the alveolar ridge, approximately 0.7-1 cm long;
2. The mucoperiosteal flap is separated from the bone;
3. Using sharp wire cutters, a part of the incisor and alveolar ridge is cut off, approximately 3-5 mm deep;
4. After that, the edges of the wound are reduced (additional suturing of the wound is not required) and a dynamic observation of the individual is established.

According to the results of clinical and laboratory studies, it was found that the developed model meets all the above criteria and gives the expected results.

At the second stage, MSCs were cultivated. Cells were extracted from a 4 × 3 × 3 mm gingival tissue sample. The sample was crushed to fragments no larger than 1 mm^3, the resulting tissue mass was incubated in 2 mL of collagenase I solution (1000 U/mL) (Sigma-Aldrich, USA) at 37 °C for 2.5 hours with continuous gentle stirring. At the end of the incubation, 10 mL of Hank's solution with 10% fetal bovine serum (FBS) (Biosera) was added to the tissue mass tube to neutralize the enzyme, after which the tissue was dissociated by actively mixing the contents of the tube.

The resulting cell suspension was transferred to a new tube, the cells were pelleted by centrifugation (1100 rpm, 10 min), resuspended in growth medium, and transferred to a T25 culture flask (Nunc, Denmark). Cells were grown in DMEM + F-12 growth medium (PanEco, Russia) supplemented with 10% FBS, glutamine (0.03%), and gentamicin (50 μg/mL medium). Cells were grown in a CO_2 incubator at 37 °C in an atmosphere with 5% CO_2.

On the 3rd day after inoculation of the primary culture, dividing cells were observed; on the 6th day, sections of the monolayer began to form. Cell reseeding was performed after the monolayer reached 80% confluence. Cells were removed from plastic using 0.25% TrypLE solution (Gibco, Thermo Fisher Scientific, USA).

Fibroblasts were used for administration to rats after the 2nd reseeding. Cells were removed from plastic, washed twice to remove TrypLE residues with Hank's solution (on the first wash with 10% FBS), after that the cells were resuspended in saline at a concentration of 1.65 million cells/mL.

The third stage included the direct conduct of an experimental study. For this, the developed model of atrophy was reproduced and the drug based on MSCs was administered according to the following steps:

1. Interrupted sutures were applied to the wound surface;
2. The introduction of the filler of the drug was carried out through the reduced edges of the wound;
3. An insulin syringe was used to administer a suspension containing MSCs in an amount of 200 μL.

Results and discussion

To evaluate the results of the experimental study and the course of the inflammatory reaction in particular, a complete blood count (CBC) was conducted. As the main indicator for evaluation, the indicators of the number of leukocytes (WBC) were taken, which were compared with the average indicators of the level of leukocytes in the intact group. Based on the results obtained, a graph was compiled (Figure 1), which shows that both in the atrophy model group and in the experimental group, a significant increase in the level of leukocytes is determined relatively to the average norm on the first day of the experiment. This indicates the development of an active inflammatory reaction. Normalization of the level of leukocytes occurs by the end of the second week of observations.

An interesting fact is that the level of leukocytes in the experimental group is higher than in the group of the developed model. This can be determined by a more intense inflammatory response mediated by the effector influence of stem cells on innate and adaptive immunity.

According to the objective examination data, it was found that visible bone loss was determined in the atrophy model group. At the same time, it was determined that the developed model does not cause the death of the neurovascular bundle of the lower incisor and, accordingly, is not capable of provoking the development of pathological processes in the tissues of the tooth and in periodontal structures (Figure 2).

By the end of 3 months, there is a significant thickening of the alveolar process of the lower jaw in the experimental group. Objectively, the thickening is dense, without pathological noises during palpation (Figure 3).

MSCs play an important role in the regeneration of tissues and organs due to the ability to differentiate and renew themselves. Due to these abilities, MSCs are the main contenders for work in the field of tissue engineering. Besides that, MSCs have a number of other features that improve implant survival. This is the ability to stimulate osteogenesis due to the release of growth factors, the immunomodulatory effect due to the production of inflammatory factors. Particular attention should be paid specifically to the immunomodulatory effect, since MSCs are able to not only prevent the immunological rejection of the graft, but also activate immune responses directly in the bone.

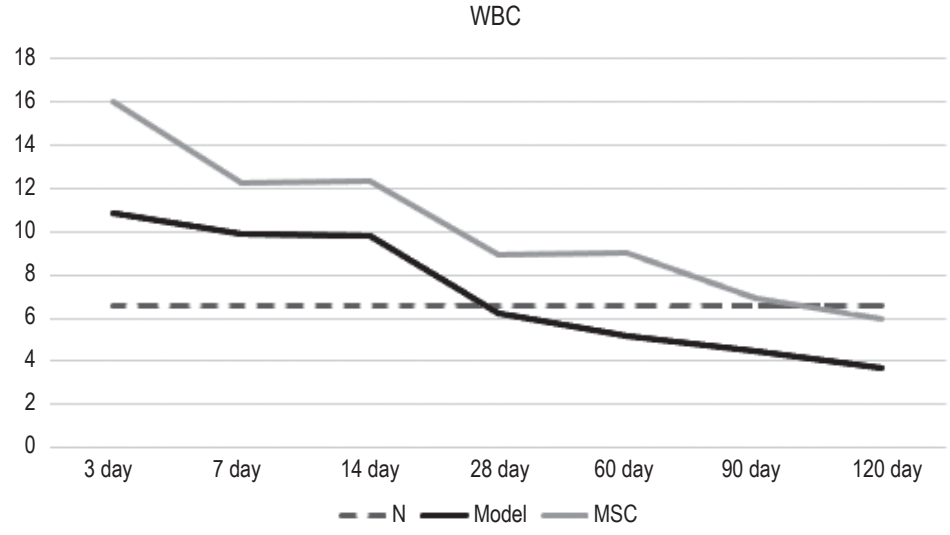

Figure 1. WBC level graph

Figure 2. Alveolar bone atrophy

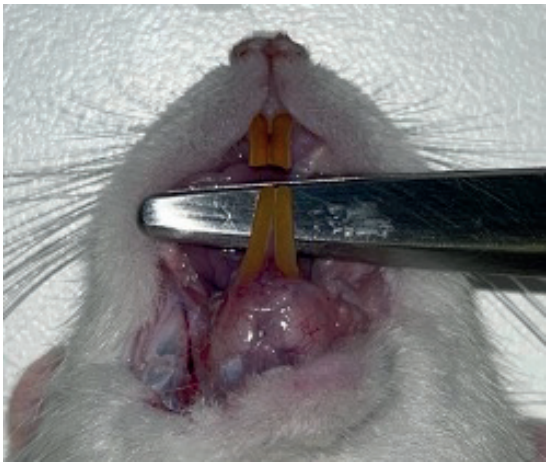

Figure 3. Newly-formed bone area

In recent years, many scientific papers have appeared, the authors of which propose methods for reconstructing bone defects by using grafts populated with stem cells or impregnated growth factors. Oftentimes, the variant of colonization by mesenchymal multipotent stem cells is described, since they have a low expression of MHC class II and CD80/CD86 molecules, due to which they have low immunoconflict and the possibility of using both autologous and allogeneic cultures. MSCs exhibit immunomodulatory effects by influencing inflammatory responses and optimizing immune cell responses. By inhibiting the proliferation and production of cytokines by antigen-presenting cells (APCs) and T lymphocytes, MSCs regulate the immunological background of bone tissue remodeling. Also, MSCs produce prostaglandin E2 (PGE2) and nitric oxide (NO), which has an immunosuppressive effect and, as a result, reduces the risk of transplant rejection. Stem cells, through a number of mechanisms, affect T cells, reducing their viability and ability to proliferate, and stimulate the proliferation of T regulatory (Treg) cells. Predominantly, this effect is mediated by CD274 molecules that are activated by IFNγ. In addition to that, MSCs suppress the proliferation of B lymphocytes, maturation of dendritic cells, and reduce the proliferation and cytotoxicity of NK cells, which together have effects on both innate and acquired (adaptive) immunity. The mediators secreted by MSCs also affect the immunomodulatory effect of these cells. These are predominantly transforming growth factor β1 (TGF-β1), prostaglandin E2 (PGE2), indolamine-pyrrole-2, 3-dioxegenase (IDO), nitric oxide (NO), and interleukin-10 (IL-10). Their produce is regulated by IFNγ, IFNα, IL-1, IL-1β [7].

MSCs are of particular value due to their antimicrobial effects. This is associated with direct and indirect mechanisms. Direct effects are directly related to the release of antimicrobial peptides by MSCs, such as cathelicidins, lipocalin-2, and β-defensins. Cathelicidins, similarly to other peptides, are active against all types of bacteria, both $Gram^+$ and $Gram^-$, against some viruses, fungi, and protozoa. To a large degree, these effects are due to the incorporation of peptides into the structure of the bacterial wall and the formation of a pore in it. In addition, this peptide functions as a chemotactic agent for neutrophils, monocytes, and T cells. With the development of the infectious process, MSCs also have an effector effect on the innate link of immunity, particularly on neutrophils and monocytes, increasing their migration to the infectious focus and enhancing their antimicrobial activity [1].

The ability of MSCs to differentiate along the osteogenic pathway has a beneficial effect on bone repair processes. This process is regulated by a number of signaling pathways, including Wnt, TGF-β, PI3K/Akt. In addition, BMP-2, BMP-6, BMP-7, and BMP-9 are important triggers for osteogenic differentiation of MSCs [2].

All of the above effects of MSCs favourably affect the regulation of the immunological mechanisms of inflammatory and regenerative processes.

Conclusion

There are many more questions about the use of MSCs as a material for guided bone tissue regeneration that require detailed study. However, the results obtained indicate the prospects for the use of this technology. Influence on the links of innate and adaptive immunity, processes of bone metabolism, allows you to fully activate the natural processes of bone repair by creating a specific microenvironment and the ability of MSCs to transform into highly differentiated cells. It is the increase in the volume of bone tissue and not the accelerated healing of the bone defect that makes it possible to conclude that atrophy is prevented.

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Поступила 11.04.2023
Отправлена на доработку 12.04.2023
Принята к печати 18.04.2023

Received 11.04.2023
Revision received 12.04.2023
Accepted 18.04.2023