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## EXPERIMENTAL SUBSTANTIATION FOR APPLYING ACTOVEGIN IN COMBINATION WITH OSTEOPLASTIC MATERIALS IN MANDIBULAR BONE TISSUE REGENERATION

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The aim of the study is to perform a comparative assessment of the effectiveness of applying various osteoplastic materials in combination with Actovegin in stimulating bone tissue regeneration. Materials and methods. In order to study the osteoplastic properties and determine the peculiarities of the course of reparative processes, as well as to substantiate the treatment method we elaborated, *in vivo* experimental studies were carried out on 36 male rabbits weighing from 1.5 to 2.5 kg at the Scientific Research Centre of the Azerbaijan Medical University. The animals were kept in a vivarium under standard conditions on the standard diet and were under the study for 60 days since the inclusion in the experimental model. Having been included into the study, the animals were randomly divided into 3 groups of 12 rabbits in each: in the animals of Group I, the main group, individual bone defects were formed on both sides of the lower jaw; the subgroup A included animals, whose artificially formed defects on one side of the jaw were closed with an allograft and platelet-rich fibrin exudate (PRF), subgroup B included animals with the same modelled damage, who received the treatment mentioned above and additional Actovegin; Group II was a comparison group, received a mixture of autogenous bone, PRF and Actovegin injected into jaw defects; Group III was the control group, which received PRF and Actovegin injected intravenously in a dose of 1 mg daily for 2 weeks. Results. Neutrophilic infiltration was found at the maximum level ( $1.33 \pm 1.07$ ) in the area of the mandibular bone defect in subgroup A of I group, where only the allograft was used without additional Actovegin. In group III, who received the combination with PRF and intravenous administration of Actovegin, the average rate of lymphocytic infiltration by the end of the experiment was  $1.75 \pm 0.75$  that correlates with the severity of the degree of bone defect closure. Vascularization or blood supply to pre-damaged bone tissue, in particular to the residual area of the bone defect, was detected at the lowest level in the group of animals, who did not receive Actovegin during the implantation of osteotropic material into the defect site ( $1.25 \pm 0.45$ ). Vascularization, which impacts the bone tissue regeneration, was the most intensive when applying Actovegin ( $1.91 \pm 0.66$ ). In the samples obtained in group II and subgroup B, more capillaries and, accordingly, a high density of the capillary network ( $p < 0.01$ ) were detected in the area of the bone defect more often than in other groups of animals. Conclusion. Inflammatory changes (neutrophilic, lymphocytic and plasma cell infiltration) in the residual zone, which is part of the bone defect, were less pronounced in the group of topical application of autologous bone and Actovegin. At the same time, it was noted that the amount of eosinophilic infiltration and granulomatous reactions were more intense when using an allograft.

Key words: rabbits, experimental model, morphology, PRF, bone graft, actovegin, bone tissue regeneration.

### Introduction

After the loss of teeth, especially with the development of inflammatory-dystrophic periodontal diseases, the dystrophy and gradually atrophies start developing in alveolar process [1]. In current dental practice, it is customary for specialists to place implants immediately after tooth extraction to shorten the rehabilitation period, prevent further atrophy, and reduce the width and height of the alveolar bone tissue [2, 3]. At the same time, despite the effectiveness of prosthetic appliances placed on implants, there can be a number of problems often associated with complications that develop due to the bone tissue loss in the peri-implant area [4, 5]. That is, only the presence of a sufficient amount of bone tissue in the region of the alveolar ridge makes the favourable prognosis for orthopedic treatment with dental implant placement. To date, dentistry has accumulated sufficient experience in the development and implementation of various osteoplastic techniques for guided tissue regeneration, in particular, in dental implantation, in order to restore the amount of the jaw bone, taking into account the specific clinical situation [6, 7].

The problem of stimulation of bone regenera-

tion, replacement of lost bone or replacement of a bone defect can be partially solved by applying bone autoplasty, but it is worth noting that, along with certain advantages, this technique has a number of disadvantages associated with additional morbidity at the donor site and the need for two operative procedures, risks of infections [8, 9]. Therefore, a rather limited spectrum of action of various drugs used to eliminate jaw bone defects necessitates the development of optimal and pathogenetically substantiated combinations of drugs that simultaneously have osteoinductive and anti-inflammatory properties [10].

### The aim of the study

The purpose of this study is to care comparative evaluation of the effectiveness of various osteoplastic materials in combination with Actovegin in stimulating bone tissue regeneration.

### Materials and methods

To investigate the osteoplastic properties and determine the peculiarities of the course of reparative processes, as well as to substantiate the treatment method elaborated, experimental *in vivo* studies were carried out on 36 male rabbits weighing

from 1.5 to 2.5 kg at the Research Center of the Azerbaijan Medical University.

The animals were kept in a vivarium on a standard diet and were under the study for 60 days since the inclusion in the experimental model. Having been included into the study, the animals were randomly divided into 3 groups of 12 rabbits in each: in the animals of Group I, the main group, individual bone defects were formed on both sides of the lower jaw; the subgroup A included animals, whose artificially formed defects on one side of the jaw were closed with an allograft and platelet-rich fibrin exudate (PRF), subgroup B included animals with the same modelled damage, who received the treatment mentioned above and additional Actovegin; Group II was a comparison group, received a mixture of autogenous bone, PRF and Actovegin injected into jaw defects; Group III was the control group, which received PRF and Actovegin injected intravenously in a dose of 1 mg daily for 2 weeks. Under general anesthesia, a 2 cm long skin incision was performed, and following the exfoliation of underlying soft tissues, the lateral surface of the lower jaw was exposed, rounded bone defects 0.5 cm in diameter and 3-4 mm deep were formed with a spherical cutter. This study was carried out in accordance with international ethical requirements and the rules adopted by the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123), Strasbourg, 1986). The animals were killed after 60 days of the experiment, and the bone

fragments of the alveolar arches with defects were removed and fixed in formalin.

The tissue samples were embedded in paraffin using an embedding device (Leica EG 1150, Germany) and blocks were obtained. 5 µm sections were made from blocks by a microtome (Leica RM 2125 RTS, Germany). The sections were stained with hematoxylin and eosin (Merck, Germany) and covered with Entellan solution in a coverslip (Leica CV 5030, Germany). The prepared micropreparations were examined using a light microscope (Leica DM 1000 LED, Germany). All changes observed during the microscopy were recorded using a microscope camera (Leica ICC 50W, Germany).

The results obtained were analyzed using the statistical software version 15.0 (SPSS 15.0) of the Statistical Package for the Social Sciences. Arithmetic mean values (M) and errors were calculated.

**Results and discussion.** A diagram representing the results of a comparative analysis of the morphological parameters recorded in the residual tissue in the area of the bone defect in different groups is shown below (Figure 1). In the study groups, most samples showed a certain and somewhat different degree of leukocyte (neutrophil) infiltration.

Neutrophilic infiltration was recorded at the maximum level ( $1.33 \pm 1.07$ ) in the area of the mandibular bone defect in subgroup A of the I group of animals, where only the allograft was used without additional use of Actovegin.

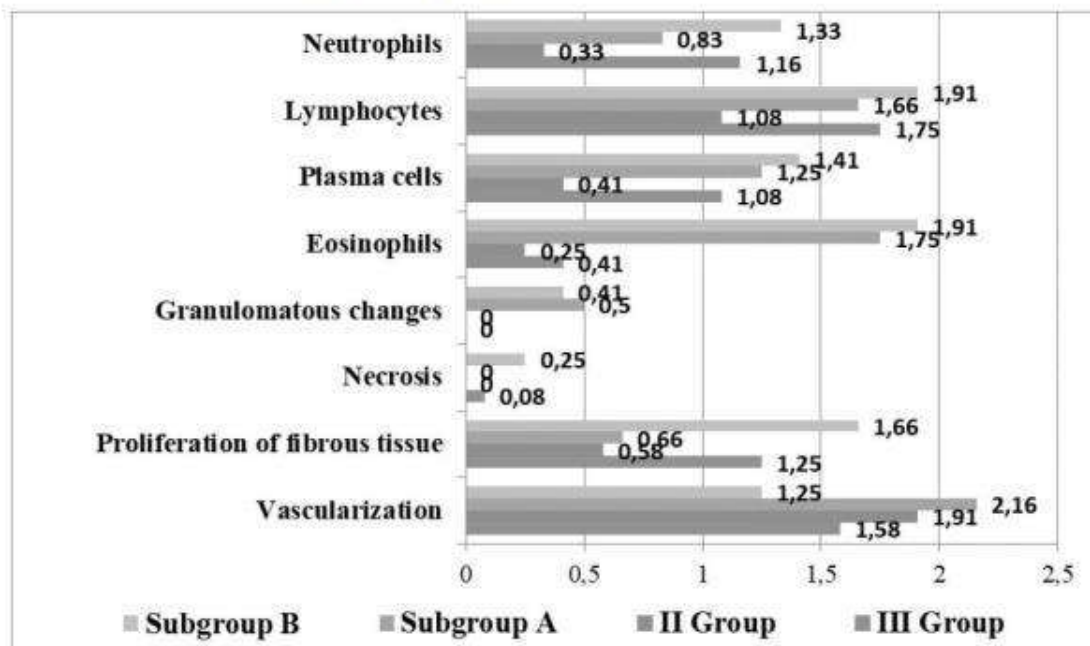


Figure 1. Average indices of morphological parameters revealed in the residual residual tissue in the area of the bone defect in different groups

In the other subgroup with the use of an antihypoxic drug, the neutrophil infiltration indices were significantly lower ( $0.83 \pm 0.83$ ). In group II, where autologous bone was used, by the end of the experiment, neutrophilic infiltration was determined at the lowest level ( $0.33 \pm 0.49$ ), and in this group of animals, after imposition of platelet-rich fibrin (PRF) into the area of the defect and intravenous administration of Actovegin, neutrophilic infiltration was recorded. Infiltration was approximately of the same

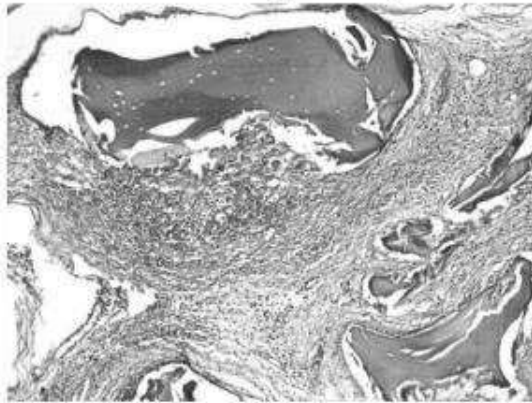


Figure 2. Density of neutrophil infiltration in the III group of experimental animals (staining: hematoxylin-eosin, magnification: x100)

Micropreparations belonging to these groups demonstrated that around the free edge of the skin wound and in the soft tissues around the surgical suture there were the largest area of structural changes in fibrous tissue with the growth of collagen and fibrous fibres. Secondary changes in the form of identified areas of local hyalinosis of small subepidermal arteries among dense fibrous tissue, which became thinner towards deeper tissues, were also determined in the samples in the area of the surgical suture belonging to the above mentioned groups.

Lymphocytic infiltration, as well as neutrophilic infiltration, was recorded at the maximum level ( $1.91 \pm 0.79$ ) during the treatment of a bone defect in subgroup A. In the other subgroups, following the Actovegin application, the indicators were relatively low ( $1.66 \pm 0.65$ ) (Figure 3). In the second experimental group, infiltration by lymphocytes in the test samples was determined at the lowest level ( $1.08 \pm 0.66$ ).

In group III, when applying the combination of PRF and intravenous Actovegin administration, the average rate of lymphocytic infiltration by the end of the experiment was  $1.75 \pm 0.75$  that corresponded to the degree of bone defect closing.

Thus, when comparing the identified indicators, there was a significantly low level of lymphocytic infiltration in the II group of experimental animals that can result from both osteoconductive and anti-inflammatory properties of the above mentioned

intensity ( $1.16 \pm 0.93$ ) as in subgroup A, in which this drug was not administered (Figure 2).

It has been found that the bone defect after transplantation, formed in group II, is characterized by a lower density of neutrophil infiltration compared to other groups ( $p < 0.01$ ). Fibrotic changes were more pronounced in the control group and in the comparison group.

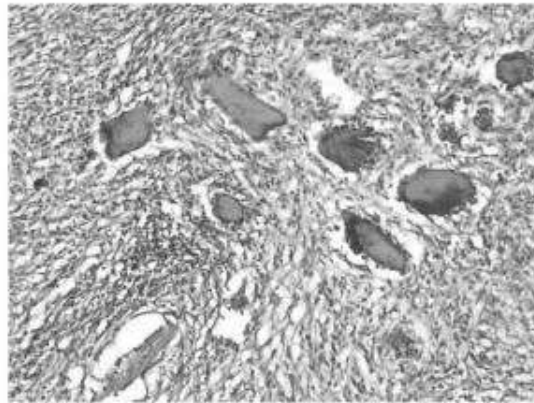


Figure 3. Lymphocytic inflammatory infiltration of the bone defect and surrounding tissues in subgroup B (stain: hematoxylin-eosin, magnification: x100)

combination of Actovegin and autogenous bone ( $p < 0.05$ ).

Histological confirmation of the most pronounced infiltration by plasma cells, representatives of the population of inflammatory cells or blood cellular elements that play an important role in the development of the inflammatory process, can be indicators recorded at the highest level in group I, especially in its subgroup A ( $1.41 \pm 0.66$ ) (Figure 4). At the same time, some similarities were found between these data and the results of studies on the detection of neutrophilic and lymphocytic infiltration in groups. Thus, in subgroup B, where Actovegin was used, plasma cell infiltration was relatively low and reached to  $1.25 \pm 0.45$ .

In group II, by the end of the experiment, infiltration with an increase in the quantitative indicators of plasma cells, as well as other studied components of the cellular inflammatory response, was recorded at the lowest level ( $0.41 \pm 0.51$ ). In group III, after the combined use of platelet-rich fibrin (PRF) and Actovegin intravenously, the average intensity of plasma cell infiltration was recorded at a mean value of  $1.08 \pm 0.66$ . Thus, when comparing the obtained data, it was found that a mixture of autogenous bone, PRF and Actovegin produces the most favourable effect on the inflammatory process and inhibits an intensity of the inflammatory reaction with a predominance of plasma infiltration, against the background of an increase in the activity of representatives of the normal cell population.

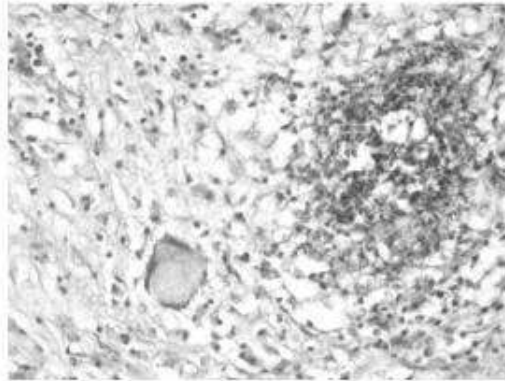


Figure 4. A small number of plasma cells with mixed inflammatory infiltration in the residual zone of a bone defect in subgroup A (stain: hematoxylin-eosin, magnification: x200)

High rates of density and frequency of eosinophil infiltration that play an important role in maintaining the inflammatory process were recorded in the area of artificial bone defect in subgroup A (Figure 5).

Eosinophilic infiltration of a slightly lower intensity was determined in subgroup B, where, in order to enhance the reparative process in the bone defect, a drug with an antihypoxic effect was additionally used. In group II, for which the autologous bone was used, eosinophilic infiltration was found at the lowest level ( $0.25 \pm 0.45$ ).

Summarizing the data obtained in all experimental groups and comparing them, we can state that rare cases of intense eosinophilic infiltration were observed only in a few samples, more precisely in group III, where, along with PRF, Actovegin was administered intravenously, and a similar pattern was observed in the II group of experimental animals ( $0.41 \pm 0.51$ ). When comparing the indicators, it was found that the intensity of eosinophilic infiltration was significantly higher in group I ( $p < 0.001$ ).

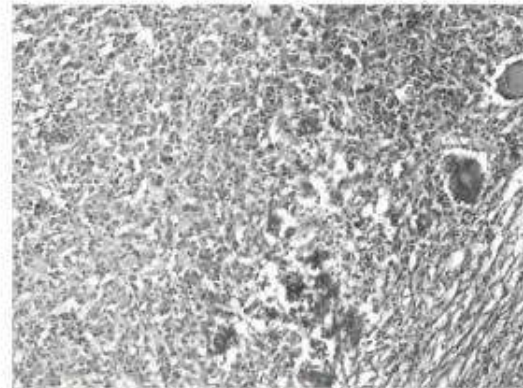


Figure 5. Eosinophils with mixed infiltration in the area of residual bone tissue in group I (staining: hematoxylin-eosin, magnification: x200)

Granulomatous changes, whose dominant histological sign is the presence of granulomas, and associated histiocytes, collected in tissue complexes, were found in all biological samples without exception taken in the I group animals, where the above disorders occurred with the same intensity in both subgroups ( $0.50 \pm 0.67$  and  $0.41 \pm 0.66$ ) (Figure 6). In groups II and III, a similar degree of progress of histiocytic infiltration and granulomatous reaction with the participation of cells of the macrophage system was not observed, and when comparing the average values between group I, groups II, and group III, statistically significant differences were found ( $p < 0.05$ ). The presence of only individual foci of residual tissue necrosis in the area of the bone defect was detected in several samples in I group animals, which did not receive Actovegin ( $0.25 \pm 0.45$ ), and in the III group, where a combination of platelet-rich fibrin (PRF) and Actovegin administered intravenously ( $0.08 \pm 0.28$ ).

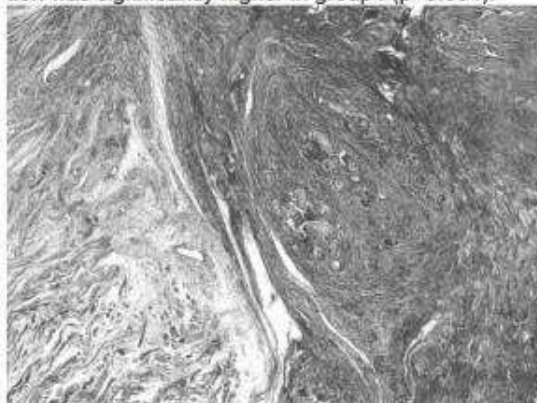


Figure 6. Granulomatous reaction around the allograft in the histological material taken from animals of the 1st group (stain: hematoxylin-eosin, magnification: x40)

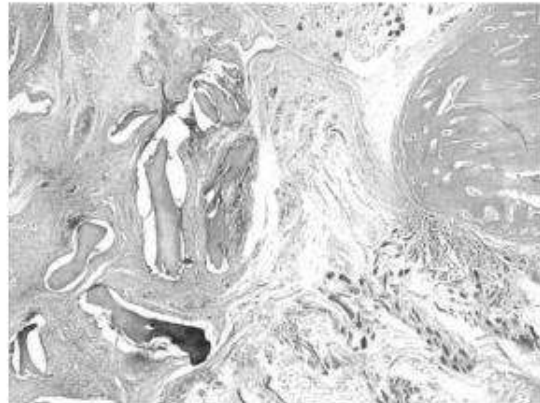


Figure 7. Growth of fibrous tissue in the bed of the bone defect, neoplasm of bone tissue in the fibrous tissue and rarefied inflammation in the group III preparation (stain: hematoxylin-eosin, magnification: x40)

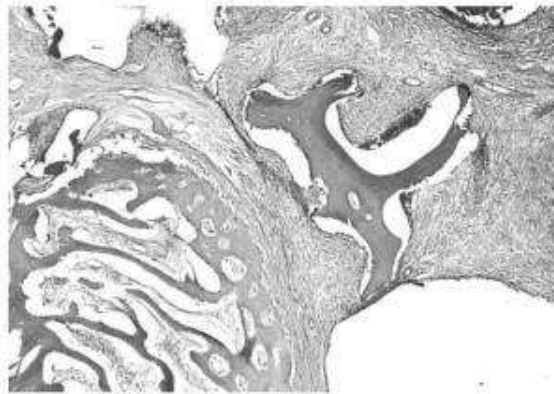


Figure 8. Intense vascularization in the area of the bone defect in a biomaterial sample from the II experimental group (staining: hematoxylin-eosin, magnification: x100)

In other groups of animals, necrosis was not observed in any of the samples. At the same time, after the processing of the findings, there was no statistically significant difference in the average values of this parameter in the experimental groups ( $p > 0.05$ ). Proliferating fibrous tissue, mainly with insufficient oxygen supply to organs and tissues was recorded at the highest level in the bone defect in animals of subgroup A, which did not receive Actovegin ( $1.66 \pm 0.88$ ), while in the opponent subgroup, the use of an antihypoxic drug significantly reduced the intensity of fibrous tissue proliferation, to the values of  $0.66 \pm 0.65$  ( $p = 0.004$ ).

The lowest rates were detected by the end of the experiment in the II and III groups,  $0.58 \pm 0.51$  and  $1.25 \pm 0.62$ , respectively (Figure 7). Thus, the bone defects formed in group II and in subgroup B were the least susceptible to the formation of proliferating fibrous tissue ( $p < 0.01$ ).

Vascularization or blood supply to pre-damaged bone tissue, in particular to the residual area of the bone defect, was recorded at the lowest level in the group of animals where Actovegin was not used during the implantation of osteotropic material into the defect zone ( $1.25 \pm 0.45$ ). Vascularization, on which the volume of regenerating bone tissue depends to a certain extent, was most intensive when using Actovegin ( $1.91 \pm 0.66$ ) (Figure 8). In the samples obtained in group II and subgroup B, more capillaries and, accordingly, a high density of the capillary network ( $p < 0.01$ ) were detected in the area of the bone defect more often than in other groups of animals.

Thus, pronounced vascularization, against the background of abundance or the formation of new blood vessels in the tissue, enhances the blood supply to the bone residual cavities and the muscles surrounding it, and thus allows us to speak about the intensification and acceleration of regenerative processes when filling artificially created bone defects by us with a combination consisting of platelet-rich fibrin (PRF), autogenous bone (or al-

lograft) and Actovegin.

Based on the morphological parameters analyzed above, it seems clear that more satisfactory results were observed in the experimental group with combined and local application of autogenous bone and Actovegin. It was after the use of Actovegin in bone defects that the intensity of morphological changes associated with hypoxia significantly decreased.

### Conclusions

When determining the intensity of integration and the influence of a combination of various materials, including Actovegin, after closing a bone defect, by the peculiarities of bone tissue regeneration, we observed some morphological changes in the area of a bone defect formed in the lower jaw of experimental animals:

Local application of Actovegin in the allograft group did not affect the formation of trabecular and newly formed bone tissue, but, at the same time, a comparatively smaller area of residual residual bone tissue was found in the area of the bone defect with a statistically significant difference.

Inflammatory changes (neutrophilic, lymphocytic and plasma cell infiltration) in the residual zone, which is part of the bone defect, were less pronounced in the group of topical application of autologous bone and Actovegin. At the same time, it was noted that the amount of eosinophilic infiltration and granulomatous reactions were more intense when using an allograft.

Prospects for further research consist in the clinical and laboratory study of the effectiveness of Actovegin in combination with osteopathic materials in dental implantation.

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### Реферат

ЕКСПЕРИМЕНТАЛЬНЕ ОБГРУНТУВАННЯ ЗАСТОСУВАННЯ АКТОВЕГІНА В КОМБІНАЦІЇ З КІСТКОВОПЛАСТИЧНИМИ МАТЕРІАЛАМИ В РЕГЕНЕРАЦІЇ КІСТКОВОЇ ТКАНИНИ НИЖНЬОЇ ЩЕЛЕПИ

Хефзоллесан С.М., Мамедов Ф.Ю., Архмамедова Г.М., Мамедов Р.М.

Ключові слова: кролі, експериментальна модель, морфологія, PRF, трансплантат кістковий, актовегін, регенерація кісткової тканини.

**Метою** дослідження була порівняльна оцінка ефективності застосування різних остеопластичних матеріалів у поєднанні з Актовегіном у стимульованій регенерації кісткової тканини. *Матеріал та методи.* З метою вивчення остеопластичних властивостей та визначення особливостей перебігу репаративних процесів, а також для обґрунтування запропонованого нами методу лікування на базі Науково-дослідного центру Азербайджанського медичного університету було проведено експериментальні дослідження *in vivo* на 36 кроликах-самцях вагою від 1,5 до 2,5 кг. Досліджуваних тварин утримували у віварії з нормальною дієтою, і після створення експериментальної моделі спостерігали протягом 60 днів. Після створення експериментальної моделі тварини були рандомізовані на 3 групи по 12 кролів у кожній: I (основна) група - у кожного з 12 кролів були сформовані окремі кісткові дефекти з обох боків нижньої щелепи; при заповненні штучно створених дефектів з одного боку щелепи використовували алотрансплантат, замінений багатим на тромбоцити фібриновим ексудатом (PRF) - підгрупа А; в підгрупі В використовували ту ж комбінацію, яка додатково містила Актовегін. В II групі (порівняння) суміш аутогенних кісток, PRF і Актовегіну вводили в дефекти щелепи. В III групі (контрольній) при заповненні штучно створених дефектів нижньої щелепи використовували PRF і одночасно вводили Актовегін внутрішньовенно в дозі 1 мг щодня протягом 2 тижнів.

Результати дослідження. Нейтрофільна інфільтрація була зареєстрована на максимальному рівні ( $1,33 \pm 1,07$ ) в області дефекту кістки нижньої щелепи в підгрупі А 1 групи тварин, де використовувався тільки алотрансплантат без додаткового застосування Актовегіну. У III групі при використанні PRF та у поєднанні з внутрішньовенним введенням Актовегіну середня швидкість лімфоцитарної інфільтрації до кінця експерименту склала  $1,75 \pm 0,75$ , і відповідала за тяжкістю ступеня заповнення кісткового дефекту.

Васкуляризація або кровопостачання попередньо пошкодженої кісткової тканини, зокрема залишкової області кісткового дефекту, було зареєстровано на найнижчому рівні в групі тварин, де Актовегін не використовувався під час імплантації остеотропного матеріалу в зону дефекту ( $1,25 \pm 0,45$ ). Васкуляризація, від якої певною мірою залежить обсяг регенеруючої кісткової тканини, була найбільш інтенсивною при застосуванні Актовегіну ( $1,91 \pm 0,66$ ). У зразках, отриманих у II групі та підгрупі В, в ділянці кісткового дефекту частіше, ніж в інших групах тварин, виявлялася більша кількість капілярів і, відповідно, висока щільність капілярної мережі ( $p < 0,01$ ).

**Висновки.** Запальні зміни (нейтрофільна, лімфоцитарна та плазмоцитарна інфільтрація) у залишковій зоні, що є частиною кісткового дефекту, були менш виражені у групі місцевого застосування аутологічної кістки та Актовегіну. У той же час було зазначено, що кількість еозинофільної інфільтрації та гранулематозні реакції були більш інтенсивними при використанні алотрансплантату.