MORPHOMETRIC INDICATORS OF BONE TISSUE OF RATS WHEN USING MESENCHYMAL STEM CELLS FROM WARTON'S JELLY AGAINST THE BACKGROUND OF A FRACTURE

Abstract. Bone fractures are a serious challenge for the modern health care system and its institutions. Injuries often to persons of working age with subsequent long-term health disorders, long-term recovery, and the need for rehabilitation make this type of injury a heavy financial burden for both patients and the state, taking into account the long-term consequences. There is a separate problem regarding the significantly greater severity of the course of fractures and the number of their complications in the elderly, which is especially relevant in the conditions of the "aging Western World". In this regard, there is a need to find new methods of acceleration, improvement of the quality of treatment of especially complicated fractures. Mesenchymal stem cells are the most promising means for the treatment of various pathologies that are currently being actively researched in the world. The aim of the study was to study the morphometric parameters of bone tissue in rats when mesenchymal stem cells from Warton's jelly were used against the background of a fracture. For this, a study was performed on 64 rats that underwent a tibial fracture, 32 of which (the control group) were not subject to treatment, and the other 32 (the experimental group) were injected with mesenchymal stem cells from Wharton's crucibles into the fracture site. Subsequently, histological material for morphometric research was collected on the 7th, 14th, 21st and 28th days of the study. The analysis of the obtained indicators showed that in almost all cases, regardless of the period of study or the studied parameter, the values of the thickness of bone trabeculae, the number of contacts of trabeculae with the cortex, the average number of osteocytes on bone trabeculae, the number of empty osteocytic lacunae, the number of functionally active osteoblasts were significantly higher (p<0,01-0,05) in
the experimental group. A significantly higher (p<0.01) value of the indicator of the number of functionally active fibroblasts was found in the control group on day 21. In this way, the positive effect of mesenchymal stem cells on the treatment of fractures when administered locally to the fracture zone at all investigated observation periods was proven. For the first time, data on a reduced number of functionally active fibroblasts were revealed when using stem therapy on the 21st day of the experiment.

**Keywords:** bone trabeculae, Wharton's cells, osteocytes, morphometry, stem cells, bone fracture, rats.

Рябошапко Олег Миколайович аспірант кафедри травматології та ортопедії, Вінницький національний медичний університет ім. М. І. Пирогова, вул. Пирогова 56, м. Вінниця, 21018, тел.: (0432) 55-39-10, https://orcid.org/0000-0002-2071-0371

Фіщенко Володимир Олександрович доктор медичних наук, професор, Завідувач кафедри травматології та ортопедії, Вінницький національний медичний університет ім. М. І. Пирогова, вул. Пирогова 56, м. Вінниця, 21018, тел.: (0432) 55-39-10, https://orcid.org/0000-0002-4742-9416

**МОРФОМЕТРИЧНІ ПОКАЗНИКИ КІСТКОВОЇ ТКАНИНИ ЩУРІВ ПРИ ЗАСТОСУВАННІ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН З ВАРТОНОВИХ ДРАГЛІВ НА ФОНІ ПЕРЕЛОМУ**

**Анотація.** Переломи кісток є серйозним викликом для сучасної системи охорони здоров’я та її установ. Ураження часто осіб працездатного віку з подальшим тривалим розладом здоров’я, тривалим характером відновлення, потребою в реабілітації робить даний вид травми ще й тяжким фінансовим навантаженням як для пацієнтів так і для держави, враховуючи довгострокові наслідки. Окремо стоять проблема щодо значно більшої тяжкості перебігу переломів та кількості їх ускладнень у осіб похилого віку, що особливо актуально в умовах «старіння Західного Світу». У зв’язку з цим існує потреба у пошуку нових методів прискорення, покращення якості лікування особливо ускладнених переломів. Найбільш перспективними засобами для лікування різного роду патологій, що наразі активно досліджуються у світі є мезенхімальні стовбuroві клітини. Метою дослідження було вивчити морфометричні показники кісткової тканини щурів при застосуванні мезенхімальних стовбурових клітин з вартонових драглів на фоні перелому. Для цього було виконано дослідження на 64 щурах, яким виконували перелом великогомілкової кістки, 32 з яких (група контролю) не підлягали лікуванню,
а іншим 32 (експериментальна) вводили в ділянку перелому мезенхімальні стовбурові клітини з Вартонових драглів. В подальшому гістологічний матеріал для морфометричного дослідження відбирали на 7, 14, 21 та 28 добу дослідження. Аналіз отриманих показників показав, що в практично усіх випадках, незалежно від терміну дослідження чи досліджуваного параметру достовірно (р<0,01-0,05) більші значення товщини кісткових трабекул, кількість контактів трабекул з кортексом, середньої кількості остеоцитів на кісткових трабекулах, кількості порожніх остеоцитарних лакун, кількості функціонально активних остеобластів були в експериментальній групі. Достовірно більше (р<0,01) значення показника кількості функціонально активних фібробластів в контрольній групі виявлено на 21 добу. Таким чином доведено позитивний вплив мезенхімальних стовбурових клітин на лікування переломів при місцевому введенні в зону перелому на всіх досліджуваних періодах спостереження. Вперше виявлено дані щодо зниження кількості функціонально активних фібробластів при застосуванні стовбурової терапії на 21 добу експерименту.

Ключові слова: кісткові трабекули, вартонові драглі, остеоцити, морфометрія, стовбурові клітини, перелом кісток, щури.

Statement of the problem. Among the various traumatic events that a person encounters in the course of his routine activities, one of the most serious types of traumatic process is a bone fracture. The number and localization of bone fractures is specific and differs from age, gender, country, profession and other factors. In a study of a population of 87 million people in the United States, almost 600,000 cases of bone fractures of the upper extremity alone were found. Fractures of the distal part of the radius and ulna were the most common, and the clavicle fractures were the rarest. The study also found that fractures of the phalanx and carpal bone depended on socioeconomic status (the number decreased with increasing status) [1].

In the EU, the number of osteoporosis-related fractures is estimated at 2.7 million in 2017, with the risk of increasing to 3.3 million in 2030. In turn, this will lead to an increase in health care spending on this from 37.5 billion euros in 2017 to 27% in 2030. The loss of quality-adjusted life years is estimated as of 2017 at 1.0 million years [2]. The assessment of the economic effect of fractures on the background of osteoporosis in general is actively studied by researchers in order to forecast the burden on the economy. Thus, in China, it is estimated that the number and cost of this kind of fractures will increase by 2 times by 2035 and thus amount to almost 6 million fractures with a cost of more than 25 billion US dollars [3].

All this caused a rapid development of scientific directions aimed at improving or creating new methods of treatment of fractures. One of such methods that has already found its practical application is cell therapy using mesenchymal stem cells. These cells are capable of self-reproduction and differentiation into...
different types of cell lines – adipocytes, chondrocytes, osteocytes, smooth muscle cells, fibroblasts, hematopoietic cells [4]. Among the various sources of the body that can provide them, Wharton drags are considered the most promising at the moment. Cells obtained from this source in the umbilical cord have such necessary characteristics as an easy method of isolation, the absence of traumatic consequences for the donor, and a significant differentiation potential for all cell lines [5].

Considering the ability of mesenchymal stem cells to proliferate into osteocytes, and considering the posed problem in the treatment of fractures, there is a need to conduct experimental and clinical studies in order to verify the effectiveness of the use of mesenchymal stem cells, the source of which is Wharton jelly for the treatment of fractures of the lower extremities.

**Connection of the publication with planned scientific research works.** The article is a fragment of a research topic of the Department of Traumatology and Orthopedics of the National Pirogov Memorial Medical University, Vinnytsya "Improvement of methods of diagnosis, treatment and rehabilitation of patients with injuries and diseases of the musculoskeletal system" state registration number 0123U102765.

**The purpose of the article** – to investigate the morphometric parameters of bone tissue in rats when using mesenchymal stem cells from Warton's gills against the background of a tibial fracture in rats.

**Research objects and methods.** To achieve the goal, we conducted a study on 64 Wistar rats kept under standard conditions with free access to food and water in the vivarium of the National Pirogov Memorial Medical University, Vinnytsya. All studied rats were simulated tibial fracture by osteotomy using intramedullary osteometallosynthesis. Rats were divided into two equal groups of 32 individuals each. The first group consisted of rats without treatment - the control group. The second group, experimental, in which the suspension of mesenchymal stem cells of Wharton's umbilical cord cells was injected into the fracture area of rats using a syringe.

Mesenchymal stem cells were obtained from Wharton cells of the umbilical cord from healthy donors, 39-40 weeks of gestation, delivery without pathology. All procedures took place after obtaining informed written consent, in maternity hospital No. 5 of Kyiv. Cells were isolated by the explantation method [6]. Starting with passage 1, mesenchymal stem cells from Wharton's agar were transplanted to the second passage, which was subsequently used for administration to animals. A set of surface markers was used to confirm that cells belong to mesenchymal stem cells [7].

Tissue samples from the fracture site were taken for histological examination on the 7th, 14th, 21st and 28th days of the experiment. Material for histological examination, namely bone fragments of tibiae, was fixed with 10% neutral formalin, after which decalcification of bone tissue was carried out using TRILON B, dehydrated in concentrated alcohols and immersed in paraffin. Sections obtained on
a sled microtome were stained with hematoxylin and eosin, placed on glass slides. Microscopy of histological preparations was carried out with the help of a light microscope OLIMPUS BX 41 (MoH of Ukraine State Registration Certificate No. 8120/2008, code 9011800000) using magnifications of 40, 100, 200, and 400 times. Image visualization and morphometry were performed using the morphometric program Quickphoto micro 2.3 (license agreement No. 925113924), which allows for 2737 pixels. When conducting a morphometric study, we took into account such indicators as: thickness of bone trabeculae, the number of contacts of trabeculae with the cortex, average number of osteocytes on bone trabeculae, number of empty osteocytic lacunae, number of functionally active osteoblasts.

Statistical processing of the obtained data was carried out in the license package "Statistica 6.0".

All experiments on animals were carried out in compliance with the ethical principles of the European Convention for the Protection of Vertebrate Animals.

**Presentation of the main material.**

**Research results and their discussion.** The data presented in Table 1 indicate that, compared to the control group, there was a slight increase in the thickness of bone trabeculae (by 13.2%), an increase in the number of osteocytes on their surface (by 19.8%) and contacts of bone trabeculae with the cortex (by 47.9%) relative to the femur fracture site of intact animals. In the comparison of indicators of cancellous bone tissue in the tibia, where the fracture was simulated, the increase in indicators was 17.9%, respectively.

The average number of osteocytes on bone trabeculae in the experimental group was 21.9% higher. The number of empty osteocytic lacunae is 25.7% higher. The number of functionally active osteoblasts was 25.3% higher in the experimental group, which is evidence of more active fracture healing and osteogenesis in the experimental group on the 7th day compared to the control group.

**Table 1.**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
<th>Experiment</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of bone trabeculae (nm)</td>
<td>48,36±1,90</td>
<td>55,72±2,62</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>The number of contacts of trabeculae with the cortex (un.)</td>
<td>2,092±0,108</td>
<td>4,020±0,351</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Average number of osteocytes on bone trabeculae (un.)</td>
<td>23,49±2.47</td>
<td>30,10±1,92</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Number of empty osteocytic lacunae (un.)</td>
<td>2,646±0,134</td>
<td>3,562±0,492</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Number of functionally active osteoblasts (un.)</td>
<td>12,29±2,36</td>
<td>16,46±1,28</td>
<td>&lt;0,01</td>
</tr>
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</table>

The data shown in Table 2 indicate that there is a significant increase in the thickness of bone trabeculae (by 15.6%), an increase in the number of osteocytes on
their surface (16.5%) and contacts of bone trabeculae with the cortex (by 16.6%) compared to the tibial bones of intact animals.

The average number of osteocytes on bone trabeculae in the experimental group was 16.5% higher. The number of empty osteocytic lacunae is 28.7% higher. The number of functionally active osteoblasts was 31.5% higher in the experimental group. All these indicators are evidence of even more active fracture healing and osteogenesis in the experimental group on the 14th day compared to the control group.

The data in Table 3 indicate that on the 21st day of the experiment there was a significant increase in the thickness of bone trabeculae (by 15.5%), an increase in the number of osteocytes on their surface (by 33.9%) and contacts between bone trabeculae and the cortex (by 17.5%) relative to the femur of intact animals.

The average number of osteocytes on bone trabeculae in the experimental group was 33.9% higher. The number of empty osteocytic lacunae is 29.7% higher. The number of functionally active osteoblasts was 33.6% higher in the experimental group. All these indicators are evidence of even more active fracture healing and osteogenesis in the experimental group on the 21st day compared to the control group.

### Table 2.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
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<th>p</th>
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<tbody>
<tr>
<td>Thickness of bone trabeculae (nm)</td>
<td>76.36±4.08</td>
<td>90.48±6.55</td>
<td>&lt;0.01</td>
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<td>The number of contacts of trabeculae with the cortex (un.)</td>
<td>5.908±1.252</td>
<td>7.084±0.769</td>
<td>&lt;0.12</td>
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<tr>
<td>Average number of osteocytes on bone trabeculae (un.)</td>
<td>38.48±2.75</td>
<td>46.13±3.97</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Number of empty osteocytic lacunae (un.)</td>
<td>4.616±1.227</td>
<td>6.480±0.795</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Number of functionally active osteoblasts (un.)</td>
<td>16.58±2.61</td>
<td>24.22±1.73</td>
<td>&lt;0.01</td>
</tr>
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### Table 3.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
<th>Experiment</th>
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<tbody>
<tr>
<td>Thickness of bone trabeculae (nm)</td>
<td>83.51±4.03</td>
<td>98.89±3.92</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>The number of contacts of trabeculae with the cortex (un.)</td>
<td>7.732±0.553</td>
<td>9.378±1.470</td>
<td>&lt;0.03</td>
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<tr>
<td>Average number of osteocytes on bone trabeculae (un.)</td>
<td>45.26±2.99</td>
<td>68.50±3.23</td>
<td>&lt;0.01</td>
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<tr>
<td>Number of empty osteocytic lacunae (un.)</td>
<td>6.536±0.582</td>
<td>9.300±1.522</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Number of functionally active osteoblasts (un.)</td>
<td>61.60±2.99</td>
<td>46.08±3.85</td>
<td>&lt;0.01</td>
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</tbody>
</table>
A significant increase in the thickness of bone trabeculae (by 13.8%), an increase in the number of osteocytes on their surface (29.1%) and contacts of bone trabeculae with the cortex (by 24.1%) compared to the femur of intact animals was found on the 28th day of the study (table 4).

The average number of osteocytes on bone trabeculae in the experimental group was 29.1% higher. The number of empty osteocytic lacunae is 34.7% higher. The number of functionally active osteoblasts was 30.1% higher in the experimental group. All these indicators are evidence of even more active fracture healing and osteogenesis in the experimental group on the 28th day compared to the control group.

Table 4.

<table>
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<th>Indicator</th>
<th>Control</th>
<th>Experiment</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Thickness of bone trabeculae (nm)</td>
<td>96,36±3,47</td>
<td>111,9±6,0</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>The number of contacts of trabeculae with the cortex (un.)</td>
<td>10,11±1,46</td>
<td>13,32±2,69</td>
<td>&lt;0,05</td>
</tr>
<tr>
<td>Average number of osteocytes on bone trabeculae (un.)</td>
<td>55,10±4,09</td>
<td>77,79±3,17</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Number of empty osteocytic lacunae (un.)</td>
<td>9,438±0,994</td>
<td>14,46±1,69</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Number of functionally active osteoblasts (un.)</td>
<td>54,74±4,17</td>
<td>78,33±3,28</td>
<td>&lt;0,01</td>
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</table>

The use of mesenchymal stem cells, the source of which is the skin and bone marrow, causes the formation of a larger callus, improves the mechanical properties of the bone in the fracture area and shows a better radiological picture (narrowing of the fracture gap) compared to groups of experimental animals that did not undergo cell therapy [8].

Mesenchymal stem cells have been found to share common signaling pathways with immune cells that regulate cell migration. Such adhesion molecules are MCP-1 protein and CD44. The latter is crucial for the activated extravasation of T cells at sites of inflammation [9]. It is the interaction of inflammatory cells and mesenchymal stem cells that is key to successful fracture healing. The final mechanisms of the participation of macrophages in the healing process are still not known, although it is known for certain that regeneration processes in the bone can occur without their participation [10].

Another mechanism by which mesenchymal stem cells contribute to active fracture healing is overexpression of the basic growth factor of fibroblasts. Experimental studies have shown that in the studied groups of animals with excessive expression of this factor, collagen is remodeled into a mineralized callus and thus bone strength increases [11].
A review of 82 literature sources on the possibilities of using mesenchymal stem cells for the treatment of non-union fractures showed that a successful treatment result in these cases can be achieved by introducing not only mesenchymal stem cells but also bone morphogenetic proteins, VEGF, IGF and TGF-β [12].

An important factor for the successful use of stem cells in the treatment of fractures is the provision of adequate characteristics of biomaterials, such as topological structure, surface properties, and chemical composition, which ideally allow bone ingrowth and vascularization due to their osteoconductive properties [13].

Also, an important factor is the presence of concomitant diseases in the body for which cell therapy is used. Thus, it is a proven fact that the quality and quantity of the results of the treatment of fractures with stem cells on the background of diabetes decrease [14].

**Conclusions.** In the process of analyzing the morphometric parameters of the bone in the affected area, we found sure signs that the use of injection of mesenchymal stem cells from Wharton's gills allows to significantly activate the processes of reparative osteogenesis at all its stages. Of all the studied indicators, only the value of the number of functionally active osteoblasts was greater on day 21 in the control group compared to the experimental group, which may probably be a consequence of the exhaustion of osteoblasts in this period of osteogenesis or a consequence of the release of factors affecting their activation by mesenchymal stem cells.

**References:**


Література:


