PECULIARITIES OF PLANAR MICROSCOPIC PARAMETERS OF RAT BONE TISSUE IN THE FRACTURE AREA WHEN USING WHARTON'S JELLY MESENCHYMAL STEM CELLS

Abstract. Mesenchymal stem cells are one of the most promising new means of treating human diseases, as evidenced by the clinical studies that have been actively conducted in various parts of the world over the past few decades. At the same time, the issue of the possibility of using mesenchymal stem cells of various origins for the treatment of bone fractures is debatable. One of the most promising sources of raw materials for cell therapy is human umbilical cord, namely Wharton's jelly. The purpose of our study was to conduct a morphometric study of the microscopic indicators of the area in the fracture area in rats when using mesenchymal stem cells, the source of which was Wharton's jelly. The study was performed on 64 rats of the Wistar line, on which a tibial fracture was simulated, with subsequent division into control and experimental groups. Rats of the experimental group were injected with mesenchymal stem cells, the source of which was Wharton's jelly. Later, the rats were removed from the experiment on the 7th, 14th, 21st, and 28th day, with the selection of tissues from the fracture site for histological examination followed by morphometric analysis. The obtained data were processed using statistical processing methods. In all periods of observation, except for the 14th day of observation, significantly higher (p<0.01-0.02) values of planar indicators of reparative osteogenesis were found in the experimental group. On the 14th day of observation, significantly higher (p<0.01-0.02) values of one of the studied parameters were found in rats of the control group. The features of osteogenesis identified by us in the studied groups indicate the progression of reparative processes in the fracture area in both groups during the entire observation period, with a pronounced predominance in the experimental group. At the same
time, smaller and larger values of the area of bone trabeculae in the control group on the 14th day testify to the existence of a previously undescribed phenomenon of adaptation processes, which probably inhibit the activity of mesenchymal stem cells and the factors they secrete during this period of reparative osteogenesis.

**Keywords:** Wharton's jelly, fracture, morphometry, stem cells, bone.

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**ОСОБЛИВОСТІ ПЛОЩИННИХ МІКРОСКОПІЧНИХ ПОКАЗНИКІВ КІСТКОВОЇ ТКАНИНИ ЩУРІВ В ДІЛЯНЦІ ПЕРЕЛОМУ ПРИ ЗАСТОСУВАННІ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН З ВАРТОНОВИХ ДРАГЛІВ**

**Анотація.** Мезенхімальні стовбурові клітини є одним з найбільш багатообіцяючих нових засобів лікування захворювань людини, про що кажуть дані клінічних досліджень, які активно проводяться в різних куточках світу останні кілька десятиліть. Одним з найбільш перспективних наразі джерел сировини для клітинної терапії є пуповина людини, а саме Вартонові драглі. Метою нашого дослідження було провести мофрометричне дослідження мікроскопічних показників площі в області перелому у щурів при застосуванні мезенхімальних стовбурових клітин джерелом яких були Вартонові драглі. Дослідження виконано на 64 щурах лінії Вістар, на яких моделювали перелом великогомілкової кістки з подальшим розділенням на контрольну та експериментальну групу. Щурам експериментальної групи вводили мезенхімальні стовбурові клітини джерелом яких були Вартонові драглі. Надалі щури виводили з експерименту на 7, 14, 21 та 28 добу з відбором ділянки перелому на гістологічне дослідження з послідовним морфометричним аналізом. Отримані дані обробляли використовуючи статистичні методи обробки. В усі періоди спостереження окрім на 14 добу спостереження, виявлено достовірно більші (р<0,01-0,02) значення площинних показників репаративного остеогенезу в експериментальній групі. На 14 добу
Statement of the problem. Among all types of injuries, fractures are the biggest problem for modern medicine. First of all, this happens due to the increase in the frequency of occurrence of this type of injury, as well as due to the duration of the health disorder that they cause and, accordingly, the economic losses that they cause.

Data from the database analysis of Umeå University Hospital (Sweden) from 1993 to 2007 revealed more than 10,000 cases of injuries with at least 1 fracture. During this observation period, the number of fracture cases increased by 13%, which corresponded to an incidence rate of 201 fractures per 10^4 person-years [1].

Information from hospitals in Switzerland for 2009-2011 revealed 2,840 cases of fractures of long tubular bones in teenagers. In 59% of cases, these were fractures of the radius or ulna, in 21% of the humerus, in 15% of the tibia or fibula, and in 5% of the femur [2].

When analyzing data from the Medicare database, which contains data on about 2.5 million patients, more than 47,000 patients with more than 56,000 fractures were recorded in 2011. Of them, 2.5% had unfused fractures. The mortality rate for this type of injury was 4.8% [3].

At the same time, the treatment of fractures is a significant financial burden on the health care system. An analysis of fracture treatment data in more than 200,000 individuals showed that the direct costs of treating long bone fractures ranged from $3,291 for a radius bone to $12,923 for a hip fracture [4].

In this connection, there is an active search for new methods of fracture treatment. What can improve the results of treatment and reduce its cost, reduce the risk of disability. One of these promising directions is cell therapy using mesenchymal stem cells. Considerable attention of scientists is currently focused on the study of mesenchymal stem cells, the source of which is Wharton's jelly, which until recently was not considered a source of stem cells [5].

Key words: wartonovі dраглі, перелом, морфометрія, стовбурові клітини, кістка.
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 – study peculiarities of planar microscopic parameters of rat bone tissue in the fracture area when using Wharton’s jelly mesenchymal stem cells.

Research objects and methods. The study was conducted on 64 rats of the Wistar line, located in the conditions of the vivarium of the National Pirogov Memorial Medical University, Vinnytsya, which simulated a tibial fracture by osteotomy using intramedullary osteometallosynthesis. Further, the rats were divided into two equal groups of 32 rats each: a control group without treatment and an experimental group with injection of a suspension of Wharton’s jelly mesenchymal stem cells from the umbilical cord using a syringe.

Mesenchymal stem cells were obtained from Wharton’s jelly umbilical cord from healthy donors (39-40 weeks of gestation, normal delivery) after obtaining informed written consent in maternity hospital No. 5 in Kyiv. Cells were isolated by the explantation method [6]. Starting from passage 1, mesenchymal stem cells from Wharton’s jelly 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. The morphometric study consisted in studying the area of bone trabeculae, the area of intertrabecular spaces and calculating the area ratio.

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

Experiments on animals were conducted 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 the area of bone trabeculae at the fracture site in rats of the experimental group on the 7th day was 19.4% greater than similar indicators in rats of the control group. The area of intertrabecular spaces was also slightly larger than in the control group by 11.2%. The ratio of the areas of bone trabeculae and intertrabecular spaces was slightly higher (by 9.4%) compared to intact animals.

Table 1.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
<th>Experiment</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of bone trabeculae (nm²)</td>
<td>19,44±1,25</td>
<td>24,12±1,60</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Area of intertrabecular spaces (nm²)</td>
<td>63,10±1,82</td>
<td>71,11±2,70</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Area ratio</td>
<td>0,308±0,016</td>
<td>0,340±0,027</td>
<td>=0,076</td>
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Qualitative and quantitative changes were noted on the 14th day in the structural organization of the spongy part of the tibia with a diaphyseal fracture, which are confirmed by the obtained morphometric data of the studied parameters, which are presented in Table 2. As can be seen from them, all the studied indicators of the state of the bone at the fracture site rats of the experimental group were also significantly better than the indicators of intact animals.

It was established that the area of bone trabeculae at the fracture site in rats of the experimental group was 19.6% greater than similar indicators in rats of the control group. The area of intertrabecular spaces was also slightly larger than in the control group - by 14.9%.

The ratio of the areas of bone trabeculae and intertrabecular spaces was greater by 24.6% compared to intact animals.

All these indicators are evidence of active fracture healing and osteogenesis in both studied groups on the 14th day.

Table 2.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
<th>Experiment</th>
<th>p</th>
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<tbody>
<tr>
<td>Area of bone trabeculae (nm²)</td>
<td>29,45±2,41</td>
<td>23,66±2,22</td>
<td>&lt;0,02</td>
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<tr>
<td>Area of intertrabecular spaces (nm²)</td>
<td>84,36±2,95</td>
<td>99,22±7,79</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Area ratio</td>
<td>0,349±0,017</td>
<td>0,238±0,008</td>
<td>&lt;0,01</td>
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</table>
Qualitative and quantitative changes were noted on the 21st day in the structural organization of the cancellous part of the tibia with a diaphyseal fracture, which are confirmed by the obtained morphometric data of the studied parameters, which are presented in Table 3. As can be seen from them, all the studied indicators of the state of the bone at the fracture site of experimental rats groups were better than the indicators of intact animals.

Thus, the area of bone trabeculae at the fracture site in rats of the experimental group was 23.7% larger than the similar indicators in rats of the control group. The area of intertrabecular spaces was also slightly larger than in the control group - by 11.2%.

The ratio of the areas of bone trabeculae and intertrabecular spaces was greater by 14.4% compared to intact animals.

All these indicators are evidence of active fracture healing and osteogenesis in the experimental group on the 21st day in comparison with the control group.

Table 3. 

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
<th>Experiment</th>
<th>p</th>
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<tbody>
<tr>
<td>Area of bone trabeculae (nm²)</td>
<td>32,17±2,69</td>
<td>42,21±3,56</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Area of intertrabecular spaces (nm²)</td>
<td>112,2±3,6</td>
<td>126,3±4,0</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Area ratio</td>
<td>0,286±0,015</td>
<td>0,334±0,018</td>
<td>&lt;0,01</td>
</tr>
</tbody>
</table>

Qualitative and quantitative changes were noted on the 28th day in the structural organization of the spongy part of the tibia with a diaphyseal fracture, which are confirmed by the obtained morphometric data of the studied parameters, which are presented in Table 4. As can be seen from them, all the studied indicators of the state of the bone at the fracture site in rats of the experimental group were even better than those of intact animals.

The area of bone trabeculae at the fracture site in rats of the experimental group was 21.4% greater than the similar indicators in rats of the control group. The area of intertrabecular spaces was also slightly larger than in the control group - by 13.6%.

The ratio of the areas of bone trabeculae and intertrabecular spaces was greater by 9.0% compared to intact animals.

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.
Numerous studies have shown that mesenchymal stem cells are an excellent composite material for bone tissue healing, which occurs both directly as a result of cell differentiation and due to the expression of chemokine receptors [8]. It is the latter that is currently considered a key element in assessing the effectiveness of the use of stem cells for one or another type of therapy. Such receptors include CXCR-4, SDF-1, RANTES, MIP-1α, MCP-1, CCL25 and CXCL16 [9].

The use of mesenchymal stem cells in combination with other methods of therapy gives amazing results. Thus, the combination of adipose-derived mesenchymal stem cells, cancellous bone graft and chitosan hydrogel showed pronounced positive results in radiographic examination at 0, 2, 4, and 8 weeks after surgery as early as 2 weeks [10]. Morphological studies show that already on the 10th day after introduction into the fracture zone, mesenchymal stem cells differentiate into cells that form bone tissue [11]. We have not found data that would explain the slowing down (compared to the control) of the healing processes in the fracture zone with the use of mesenchymal stem cells, or similar research results in the scientific literature, however, taking into account the involvement of various body systems and pathogenetic mechanisms in the fracture healing process, it is possible assume that such a feature may be caused by the above-mentioned chemoreceptors, which do not activate at a certain stage of healing, or a certain link in the fracture healing process temporarily blocks them.

In general, mesenchymal stem cells, the source of which is the umbilical cord, have the following characteristics: they are spherical, up to 100 nm in size, and express CD9, CD63, and CD81. When introduced into the fracture zone, they have a powerful effect of enhancing osteogenic differentiation [12].

It is worth noting the peculiarities of the accumulation of mesenchymal stem cells depending on the method of administration. Thus, when administered intravenously in animal models with a fracture, their accumulation in the fracture zone remains at a high level, with the concentration remaining for at least 7 days. Healing of fractures in such cases is noted 3 weeks after the injection [13].

Thus, conducting new clinical studies on animal models is a promising direction in modern medicine, which allows revealing new data on the influence of mesenchymal stem cells on the treatment processes of various pathologies, including fractures [14].

### Table 4.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Control</th>
<th>Experiment</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of bone trabeculae (nm²)</td>
<td>42,11±3,98</td>
<td>53,62±3,85</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Area of intertrabecular spaces (nm²)</td>
<td>122,7±5,7</td>
<td>142,1±4,2</td>
<td>&lt;0,01</td>
</tr>
<tr>
<td>Area ratio</td>
<td>0,343±0,019</td>
<td>0,377±0,016</td>
<td>&lt;0,02</td>
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</table>
Conclusions. The peculiarities of the morphometric parameters in the fracture site in rats of the control and experimental groups indicated by us, indicate a significant advantage of the processes of reparative osteogenesis in the experimental group, which was injected with mesenchymal stem cells, the source of which is Wharton's dragees. At the same time, we found that on the 14th day, the indicator of the area of bone trabeculae has significantly higher values in the experimental group, which may be a consequence of the interaction of mesenchymal stem cells and the factors they secrete and adaptation processes in the fracture area.

References:


Література:


