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Microscopic changes in experimentally damaged rabbit muscles under the influence of transplanted mesenchymal stem cells

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Abstract. The relevance of the work is related to the possibility of artificially adjusting the intensity of skeletal muscle tissue regeneration in animals, which will significantly affect the tactics of rehabilitation treatment of muscle injuries in the future. The purpose of the research was to determine the effectiveness of the transplanted mesenchymal stem cells on the recovery processes in experimentally injured rabbit muscles. The method of histological examination of microscopic changes in the experimentally damaged muscle tissue of the pelvic head of the biceps femoris muscle of rabbits was used. Microscopic studies of the regeneration process of experimentally damaged striated muscle tissue established that in animals after intramuscular injection of allogeneic mesenchymal stem cells, connective tissue developed around the defect on day 2 of the experiment. On day 4, a large number of stem cells were detected in the connective tissue at the site of muscle tissue damage. On day 6, only relatively small foci of muscle tissue regeneration were observed in this area. On day 8, only small focal accumulations of stem cells were detected in this location. On the 10th day after intramuscular injection of mesenchymal stem cells, newly developed muscle fibres appeared at the site of injury. On the 14th day after intramuscular injection of mesenchymal stem cells, fully developed muscle fibres and intermuscular connective tissue were

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observed at the site of injury. In rabbits, after intramuscular injection of allogeneic mesenchymal stem cells, all regenerative processes were more active than in animals of other experimental groups and were completely completed by day 14 of the experiment. The practical value of the results obtained is to clarify the patterns of development of reparative processes in the damaged muscle area and to determine the effectiveness of using transplanted allogeneic mesenchymal stem cells in stimulating recovery processes, which is important to consider in the treatment of muscle injuries using regenerative therapy

Keywords: cell engineering; regeneration; biceps femoris; injury; skeletal muscle; histological methods

Introduction

Various sports involving animals are becoming increasingly popular in Ukraine, such as horse racing and horseback riding, bike-riding (cycling with dogs), skijoring (skiing with dogs), dog sledging, etc. Among the sports involving dogs, agility, white-pooling, dog frisbee, greyhound racing, and training of service dogs according to the international IGP testing system (a type of dog training) and mondio ring are popular.

The importance of this subject is that modern veterinary practice often encounters cases of muscle injuries among sports and working animals, namely tears, sprains and damage to the skeletal muscle tissue of the limbs. Muscle injuries are one of the most challenging problems in applied veterinary medicine, as their conventional treatment is time-consuming.

Using stem cells in the treatment of various animal diseases continues to develop rapidly. The introduction of new regenerative therapies in veterinary practice has recently attracted considerable interest (Grogan *et al.,* 2022).

Regenerative medicine is focused on restoring the functions and structure of tissues and organs using the body's biological mechanisms (El-Husseiny *et al.,* 2022).

Over the past few years, new and promising biological therapies have emerged that contribute to the therapeutic treatment of orthopaedic, inflammatory and immune processes (Dias *et al.,* 2019; Ivanovska *et al.,* 2022).

The main area of cell therapy is allogeneic mesenchymal stem cells (MSCs), which are characterised as multipotent cells of non-haematopoietic origin with the ability to self-renew and present in connective tissue (De Lima Santos *et al.,* 2019).

Stem cells – undifferentiated cells with a high capacity for self-renewal and development into tissue cells with specific functions. Thus, they are an effective therapeutic tool for improving the body's ability to repair and rebuild damaged tissues (Grogan *et al.,* 2022; McKenzie *et al.*, 2022).

Currently, mesenchymal stem cells are the most promising type of stem cells for therapeutic applications due to the ease of their isolation and cultivation, and the absence of ethical issues associated with their further use. They are becoming increasingly well-known in veterinary medicine due to their exceptional immunomodulatory properties (De Lima Santos *et al.,* 2019; Grogan *et al.,* 2022; Voga & Majdic, 2022).

The scientific literature is increasingly discussing, analysing and summarising the results of studies designed to apply allogeneic mesenchymal stem cells (MSCs) as a regenerative therapy in veterinary practice, both in experimental animals and in clinical cases (Dias *et al.,* 2019; Grogan *et al.,* 2022; Voga *et al.,* 2022).

Thus, the study and analysis of the effectiveness of innovative and promising treatment of animals with damaged muscle tissue using cellular regenerative therapy methods are quite relevant, as it can significantly accelerate the time and process of recovery of animals after injury, compared to conventional methods of treating injuries and avoiding side effects of treatment.

In experimental studies, many animal models have been used to assess the safety and efficacy of cell therapy. They were used mainly at the preclinical level to determine the prospects for further application of such methods for animal treatment (Dias *et al.,* 2019; Prišlin *et al.,* 2022). Intensive research is currently underway to expand their use in clinical settings.

Even if the mechanism of action of MSCs is not fully understood, it is known that they can interact with various types of immune cells, including T and B lymphocytes, natural killer cells, macrophages, neutrophils, and monocytes (Dias *et al.,* 2019; Prišlin *et al.,* 2022). Mesenchymal stem cells influence the innate immune system (Hoffman & Dow, 2016; Voga & Majdic, 2022).

The purpose of the research was to determine the effectiveness of mesenchymal stem cells in the restoration processes of experimental damage to the pelvic head of the biceps femoris muscle in rabbits.

Literature Review

Mesenchymal stem cells can self-renew (Romero *et al.*, 2021; Voga *et al.,* 2022) while maintaining their multipotency, as described in numerous scientific publications (Dias *et al.,* 2019). It allows obtaining the required number of cells for further clinical use, in particular, for the treatment of various diseases (Shah *et al.,* 2018; Arnhold *et al.,* 2019). For the extraction of these cells, various tissues of the adult body are most often used (Almeida *et al.,* 2010), such as bone marrow, umbilical cord, adipose tissue, placenta, and less often – amnion, tooth pulp, periosteum, etc (Kern *et al.,* 2006).

An important property of MSCs, which have been used to enhance tissue regeneration and treat animals with various diseases, is their pleiotropy, such as antiapoptosis, angiogenesis, growth factor production, antifibrosis, and the ability to migrate to the site of injury, in particular through chemotaxis (Arnhold *et al.,* 2019; Dias *et al.,* 2019).

In veterinary medicine, MSCs derived from bone marrow and amniotic fluid are often used (Barretto *et al.,* 2014; Harman *et al.*, 2016). However, the procedure for obtaining these cells usually requires surgical intervention using general anaesthesia, and, accordingly, compliance with asepsis and antisepsis rules.

Other researchers have conducted an objective macroscopic and histological assessment of the healing process of acute lesions of the rectus femoris muscle using adipose-derived stem cells. The study was conducted on 18 pelvic limbs in New Zealand rabbits, which were divided into three groups (Oh *et al.,* 2014). The results demonstrated that the addition of MSCs was accompanied by a decrease in the number of inflammatory cells in the two-week assessment (from 164.2 to 89.62 cells). Therewith, qualitative analysis of red picrosinium slides identified an increase in orange/yellow fibres in these animals, indicating the final healing process. The macroscopic evaluation demonstrated no difference between the groups. In conclusion, it is argued that using MSCs in the treatment of acute muscle injury can achieve histological advantages compared to their non-use.

However, using MSCs in clinical settings is still a challenge. Restrictions on their use in various diseases and the specifics of their application based on the type of animal hinder the development of cell-based therapies. In this research, the authors considered the possibilities of using MSCs as a powerful biopharmaceutical

agent in veterinary cellular regenerative therapy based on the available evidence, analysing the already approved experimental material of veterinary colleagues and based on the results of their research.

Materials and Methods

During the period from 2019 to 2021, experimental studies were conducted at the Centre for Cellular Technologies in Veterinary Medicine at the Academician I.O. Povazhenko Department of Surgery and Pathophysiology, which is part of the Faculty of Veterinary Medicine of the National University of Life and Environmental Sciences of Ukraine.

Using and maintaining animals for experiments complied with the requirements of the regulations of Ukraine and Directive 2010/63/EU "On the protection of animals used for scientific purposes" (Directive 2010/63/EU..., 2010), and general ARRIVE guidelines. All necessary permissions for the experiments were obtained from the Bioethics Committee of the NUBiP of Ukraine.

The experimental rabbits were kept in a separate room of the inpatient department of the Department of Surgery and Pathophysiology named after Academician I.O. Povazhenko. Each animal was assigned an individual number and kept in a separate cage. The rabbits were provided with a comprehensive diet, including "Purina" granular feed and hay. They had constant access to clean water from automatic drinkers. Sterile sawdust was used as bedding. Air exchange in the room and cage cleaning was performed twice a day. The air humidity was approximately 70-80%. The room temperature was maintained between 21 and 23 degrees Celsius. The experimental rabbits were exposed to normal conditions of light and darkness, consisting of a 12-hour cycle (Antosyuk, 2017).

Clinically healthy rabbits of the Butterfly breed were used for the study, namely: males in the amount of 105 heads, 3 months old, with a body weight of 2500-3000 kg. All animals were vaccinated with the combined prophylactic vaccine Pestorin Mormix, which is effective against rabbit myxomatosis and viral haemorrhagic disease, before the start of the study (Antosyk, 2017).

Allogeneic MSCs were cultivated according to the guidelines "Obtaining a fraction of rabbit bone marrow mononuclear cells with high proliferative activity" developed by the staff of the Department of Surgery and Pathophysiology named after Academician I.O. Povazhenko of the NUBiP of Ukraine. These cells were extracted from the red bone marrow of donor rabbits. For the extraction of red bone marrow from small animals, a method of lifetime obtaining was used, which included its selection in the area of the proximal and distal epiphyses of the humerus and femur. After shaving the hair and anaesthetising the tissues by sedating the animals, the skin was treated with a 5% iodine solution in the area of the proximal and distal epiphyses of the respective bones. At the next stage, bone marrow was aspirated using a medical needle for diagnostic puncture and spinal anaesthesia with a "Pencil" cut with a mandrel.

Cell manipulation and isolation procedures with cellular material were performed in a box of the Class II Biological Safety Training and Research Laboratory (ESCO). Further cultivation of cells was conducted in a CO_2 incubator (HERA CELL, Germany), which provided absolute humidity and 5% CO_2, and a room temperature of 37°C.

Laboratory tests were conducted in two locations: the Bald Veterinary Laboratory (Kyiv) and the Vetmedservice Training and Research Clinical Centre (NUBiP of Ukraine, Kyiv).

To determine the number of cells, a Goryaev camera and a PrimoVert microscope (Germany) with a 200x magnification were used. The total number of cells was calculated using the formula (Mazurkevich *et al*., 2010).

Cells were frozen in a Dewar vessel with liquid nitrogen (SDS-20, Ukraine). Cells were thawed in a water bath (EL 20, Poland) at 37°C.Culture media, solutions and preparations were stored in a Nord refrigerator (Ukraine) at 4°C and -18°C. Cell suspensions were centrifuged using a centrifuge (UNICO, USA). A thermostat TS-80M (Ukraine) was used to heat the solutions. Laboratory glassware was dried and sterilised in a dry heat oven NS-62A (Poland) and an air steriliser GPO-50 (Ukraine).

Before the operation with experimental reproduction of muscle tissue damage, rabbits were kept on a fasting diet for 12 hours. The operation was performed under general anaesthesia by intramuscular injection of a 2% solution of Xyla at a dose of 0.15 mL/kg of animal body weight. The rabbit was fixed on the operating table, and the access to the surgical field was freed by shaving the hair with an electric clipper and treated with Coutasept F solution.

Before the rabbit was put into general anaesthesia, an anticholinergic agent that blocks peripheral cholinergic systems was administered intramuscularly, and atropine sulfate at a dose of 0.05 mg/kg of animal body weight was used to prevent bronchospasm and laryngospasm, reduce glandular secretion, reflex reactions, and side effects caused by vagus nerve excitation. Then, "Zoletil" 100 was administered intramuscularly at a dose of 8 mg/kg of animal body weight.

Before recreating the muscle tissue injury, infiltration anaesthesia was performed at the access site with 0.5% novocaine solution (dose 3 mL/head) in the planned part of the modelled defect. The muscle tissue injury was reproduced by dissecting the skin and fascia, and then a 1.5x1.5 cm x1.5 cm section of the pelvic head of the biceps femoris muscle was cut off in the midplane area, as demonstrated in Fig. 1.

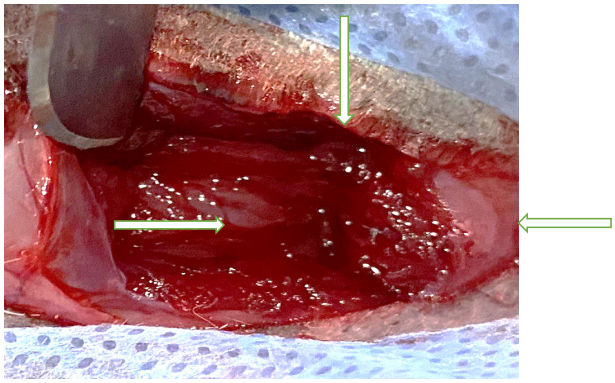

Figure 1. General view of the modelled muscle tissue defect
***Notes**: indicated by arrows; damage size 1.5x1.5 cm, 1.5 cm deep)*

The animals in the third experimental group were prescribed conventional treatment of the injury, which included two methods:

1.Surgical method: the tear in the muscle tissue was sutured with two knotted sutures. In addition, the fascia and skin were sutured with synthetic absorbable suture material Vicryl (Vicryl, Belgium). The muscle tissue was sutured with absorbable material (Chirasorb braided) (Sun *et al.*, 2018).

2. Medical method: after wound closure, 0.5 mL of Dermabond surgical glue was applied to the wound site. In addition, antibiotics were administered intramuscularly: ceftriaxone at a

dose of 0.2 mL/kg body weight once daily for 5 days and tylosin 5% at a dose of 6 mL/animal once daily for 5 days (Sun *et al*., 2018).

Animals in the fourth experimental group (control) were injected intravenously with a 0.9% sodium chloride solution. Additionally, a fifth group was established – intact animals.

After the operation, the rabbits were provided with complete peace of mind, proper housing, feeding and watering conditions. Clinical examination of animals was conducted throughout the study period.

Samples of biological material for laboratory studies were taken at baseline (before the experiment) and on days 4, 7, 10, 14, 21 and 28 of the experiment.

To obtain blood samples for further laboratory testing, a jugular vein was punctured and fresh blood was collected in tubes with an anticoagulant (Mazurkevich *et al*., 2010).

At a specific stage of the experiment, 3 animals from each experimental group were euthanised after deep anaesthesia. It was done to obtain samples of blood and muscle tissue for laboratory tests (Kern, 2006; Mazurkevich *et al*., 2010).

In the veterinary laboratory "Bald" (Kyiv), biochemical studies were performed by the spectrophotometric method using a biochemical analyser RT-9600 (China). For this purpose, commercial kits from the company "Filisit-Diagnostics" were used according to the attached instructions. Histological studies were performed at the Kasyanenko Department of Anatomy, Histology and Pathomorphology of Animals at the National University of Life and Environmental Sciences of Ukraine.

For fixation of the muscle samples, 10% formalin was used, and then the samples were dehydrated in alcohols of increasing concentration. The material was embedded in a homogenised paraffin mixture from Histomix. A sledge microtome MS-2 (Ukraine) with a section thickness of 10 and 15 μm was used to cut the blocks, according to previous studies, in particular Kern *et al.* (2006), Silva *et al*. (2010) and Sun *et al*. (2018).

For staining muscle tissue, the Van Gieson method with Karatzi's haematoxylin and eosin was used, and the preparations were placed in polystyrene. Then, the preparations were examined under an Axioskop-40 microscope (Carl Zeiss, USA) with a total magnification of 50, 100 and 400 times. Photographs of the preparations were taken using an SEO digital camera.

Morphometric parameters, such as muscle fibre thickness and interfibrillar space, were measured using the VideoTest Morphology 5.0 software. Statistical analysis of all the results was performed using the methods of analysis of variance in Statistica 10.0 software from Statsoft Inc.

Student's t-test was used to assess statistically significant differences between sample means. Differences with $P<0.05$ were considered statistically significant, which is a common practice in biological research (Mazurkevich *et al.,* 2010; Sun *et al*., 2018).

Results and Discussion

Histological studies of the regeneration of experimentally damaged striated muscle tissue in animals of experimental group 1 after intramuscular injection of allogeneic MSCs showed that on day 2 of the experiment, connective tissue was already developing around the experimentally established defect (Fig. 2). Stem cells were detected in this tissue, and in some places, a small number of these cells (2 to 4) were fused.

Evidently, intramuscular injection of stem cells enters the site of injury to a greater extent than intravenous injection, and their number in the injured area is significantly higher after local injection than after intravenous injection. In addition, significant activation of regenerative processes is documented by the presence of a large number of red blood cells in the defect area. It is assumed that the latter locally

provides significant oxygen requirements for actively functioning cells.

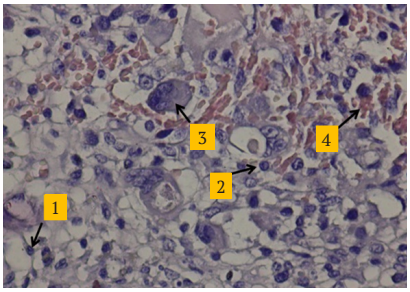

Figure 2. Average part of the experimentally developed defect on day 2 after intramuscular injection of allogeneic MSCs

Notes: *1 – fibrocyte; 2 – stem cell; 3 – stem cell fusion; 4 – normochromic red blood cells. Karatsi hematoxylin and eosin, ×400*

On day 4 of the experiment, a large number of stem cells were found in the connective tissue between muscle fibres and the establishment of new muscle fibres at the site of injury. However, a small number of partially destroyed muscle fibres were still found locally (Fig. 3).

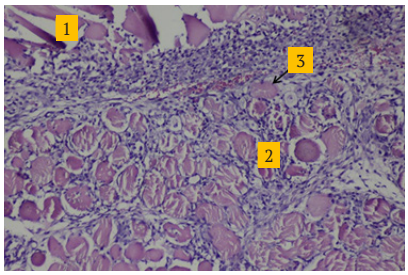

Figure 3. Average part of the experimentally developed defect on day 4 after intramuscular injection of allogeneic MSCs

Notes: *1 – partially destroyed muscle fibres; 2 – stem cells in fibrous connective tissue between muscle fibres; 3 – development of a new muscle fibre. Karatsi hematoxylin and eosin, ×50*

On the 6th day of the experiment, only relatively small foci of muscle tissue regeneration were detected at the site of injury. Most of the artificially established defect at this time was already filled with newly developed, well-developed muscle fibres, although in some places between the muscle fibres, there were still clusters of stem cells (Fig. 4).

On the 8th day of the experiment, only small focal clusters of stem cells were found at the site of injury, in which the processes of establishment of new muscle fibres were still observed. The entire area of the injury was filled with newly developed muscle tissue, which was still unevenly swollen, and the sarcoplasm of muscle fibres was pale and unevenly stained with eosin. The nuclei were dominated by heterochromatin (Fig. 5).

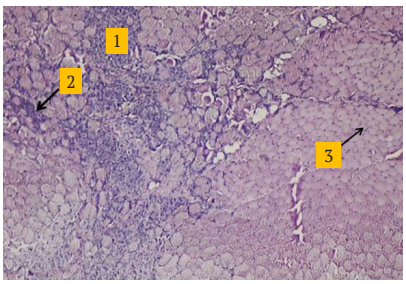

Figure 4. Average part of the experimentally developed defect on day 6 after intramuscular injection of allogeneic MSCs

Notes: *1 – focus of muscle tissue regeneration; 2 – stem cells between muscle fibres; 3 – newly developed muscle tissue. Karatsi hematoxylin and eosin, ×50*

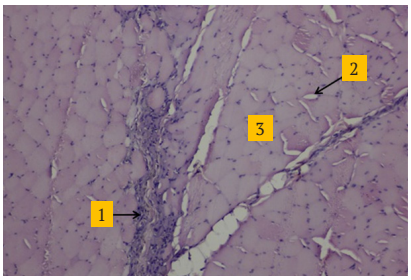

Figure 5. Average part of the experimentally developed defect on day 8 after intramuscular injection of allogeneic MSCs

Notes: *1 – focus of stem cell accumulation; 2 – oedema; 3 – newly developed muscle tissue. Karatsi hematoxylin and eosin, ×50*

On the 10th day after intramuscular injection of stem cells, only newly developed muscle fibres were detected at the site of injury. However, some of them were still incompletely developed. The muscle tissue, in general, did not vary from that of intact animals but was still slightly swollen (Fig. 6).

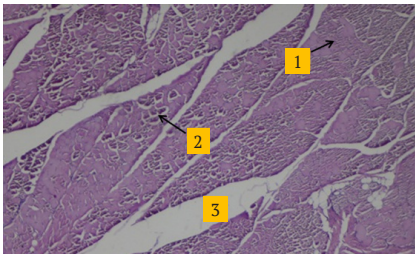

Figure 6. Average part of the experimentally developed defect on day 10 after intramuscular injection of allogeneic MSCs
Notes: *1 – established newly developed muscle fibres; 2 – incompletely established newly developed muscle fibres; 3 - oedema. Karatsi hematoxylin and eosin, ×50*

On the 14th day after the intramuscular injection of stem cells, fully developed muscle fibres and intermuscular connective tissue were observed at the site of injury, and the degree of oedema was significantly reduced compared to the previous stage of the study (Fig. 7).

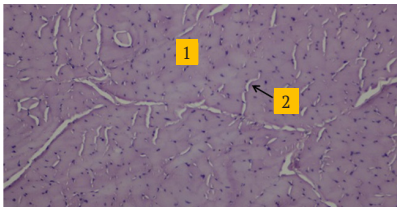

Figure 7. Average part of the experimentally developed defect on day 14 after intramuscular injection of allogeneic MSCs
Notes: *1 – newly developed muscle fibres; 2 – oedema. Karatsi hematoxylin and eosin, ×50*

Thus, in animals of experimental group 1, after intramuscular injection of allogeneic MSCs, all regenerative processes were more active compared to those in animals of other experimental groups and were completely completed by day 14 of the experiment.

In animals of experimental group 2, which were injected with allogeneic MSCs intravenously, only newly developed connective tissue was detected around the site of injury on day 2. The defect itself at this time was noticeably narrower than in animals of the control group and the group of rabbits under conventional treatment, and did not contain destroyed tissue and haemorrhages in the area surrounding the defect (Fig. 8). Instead, some of the muscle fibres around the newly developed connective tissue were destroyed, and the muscle tissue itself was slightly swollen.

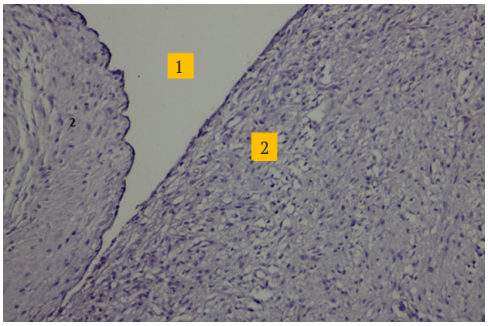

Figure 8. Upper part of the experimentally established defect on day 2 after intravenous injection of allogeneic MSCs
Notes: *1 – defect site; 2 – newly developed connective tissue. Karatsi hematoxylin and eosin, ×50*

The newly developed connective tissue contained a large number of fibrocytes, fibroblasts and collagen fibre bundles, which designed a fairly dense network (Fig. 9).

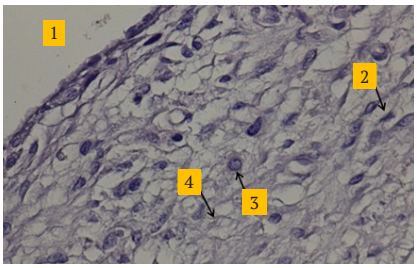

Figure 9. Upper part of the experimentally established defect on day 2 after intravenous injection of allogeneic MSCs

Notes: *1 – defect site; 2 – fibroblast; 3 – fibrocyte; 4 – collagen fibre bundles. Karatsi hematoxylin and eosin,×50*

On day 4, when stem cells were injected intravenously, small clusters of stem cells were found in the damaged and swollen muscle tissue around the defect site (Fig. 10). These stem cells were differentiated from satellite cells and leukocytes, which could theoretically infiltrate the connective tissue between muscle fibres. Satellite cells had oval or spindle-shaped nuclei and were localised on the surface of the muscle fibre. Stem cells were located in the connective tissue between the muscle fibres and were not associated with these fibres.

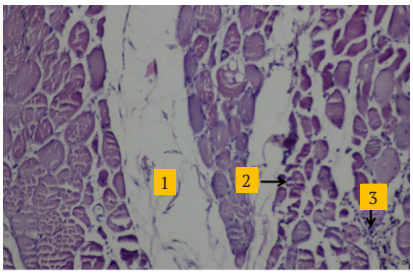

Figure 10. Average part of the experimentally established defect on day 4 after intravenous injection of allogeneic MSCs

Notes: *1 – oedema; 2 – damaged muscle fibres; 3 – stem cells in the swollen connective tissue between damaged muscle fibres. Karatsi hematoxylin and eosin, ×50*

Stem cells differed from leukocytes in their structure. They had small, intensely coloured nuclei and an extremely narrow cytoplasmic rim, which is typical of undifferentiated cells. Unlike lymphocytes, their nuclei were polymorphic (Fig. 11).

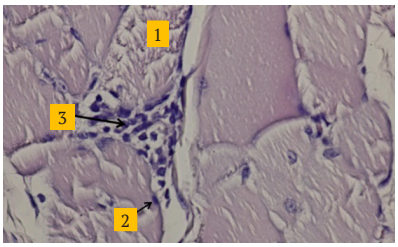

Figure 11. Average part of the experimentally established defect on day 4 after intravenous injection of allogeneic MSCs

Notes: *1 – damaged muscle fibre; 2 – satellite cell; 3 – stem cells. Karatsi hematoxylin and eosin, ×50*

On the 6th day after the injury, intravenous injection of stem cells in the area of injury demonstrated an increase in the number of stem cells in the connective tissue between muscle fibres and the presence of distinct processes of muscle tissue regeneration (Fig. 12).

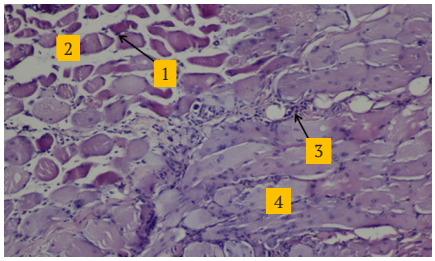

Figure 12. Average part of the experimentally established defect on day 6 after intravenous injection of allogeneic MSCs

Notes: *1 – damaged muscle fibres; 2 – oedema in the area of damaged muscle fibres; 3 – stem cells in the connective tissue between muscle fibres. Karatsi hematoxylin and eosin, ×50*

Histological studies demonstrated the process of muscle tissue development by stem cells. Initially, these cells fused to develop syncytia with a common cytoplasm. In cross-sections, these structures were round and oval in shape. Then there was a gradual increase in the volume of the common cytoplasm (Fig. 13).

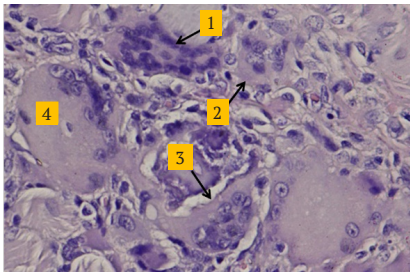

Figure 13. Average part of the experimentally established defect on day 6 after intravenous injection of allogeneic MSCs

Notes: *1 – a fusion of stem cells to establish an oval-shaped structure; 2 – a fusion of stem cells to establish a rounded structure; 3 – the initial stage of cytoplasmic volume accumulation in the cell syncytium established by stem cell fusion; 4 – a distinct increase in the volume of the common cytoplasm in the structure established by stem cell fusion. Karatsi hematoxylin and eosin, ×50*

The longitudinal section demonstrates that thin fibres of muscle-like tissue are established at first, followed by a distinct thickening of these newly developed fibres, and satellite cells appear on their surface (Fig. 14).

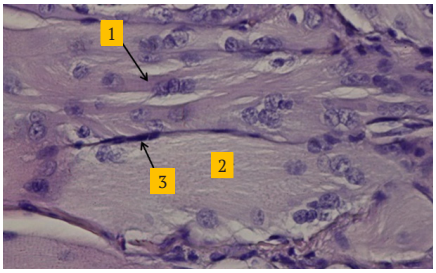

Figure 14. Average part of the experimentally established defect on day 6 after intravenous injection of allogeneic MSCs

Notes: *1 – thin fibre, similar to muscle tissue; 2 – distinct thickening of the newly developed fibre; 3 – satellite cell. Karatsi hematoxylin and eosin, ×400*

However, at this stage of the regenerative process, the newly developed fibres were still undifferentiated – they did not have the microscopic structure characteristic of striated muscle tissue.

On the 8th day after the injury, intravenous injection of stem cells in the area of injury demonstrated the presence of a large number of newly developed muscle fibres and a clear infiltration of connective tissue layers between the newly developed muscle fibres by stem cells. Cellular syncytia, which occurred on day 6 after the injury, were no longer detected. These changes indicated a high rate of the regenerative process (Fig. 15). However, the sarcoplasm of the newly developed muscle fibres still had a heterogeneous density, was vacuolated in some places, transverse striations were not detected, and heterochromatin significantly prevailed in nuclei, which indicated intensive processes of substance synthesis, i.e., the continuation of the processes of muscle fibre development (Fig. 15).

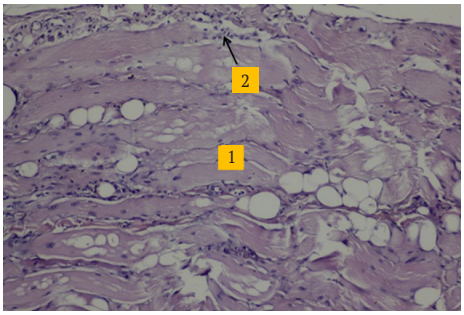

Figure 15. Average part of the experimentally established defect on day 8 after intravenous injection of allogeneic MSCs

Notes: *1 – newly developed muscle fibres; 2 – stem cell infiltration of connective tissue layers between newly developed muscle fibres. Karatsi hematoxylin and eosin, ×50*

On the 10th day after the injury with intravenous injection of stem cells, microscopic changes in the area of injury were similar to those on the 8th day, except for the absence of sarcoplasmic vacuolation of the newly developed muscle fibres.

On the 12th day after the injury, only small foci of the regenerative process and a small number of incompletely developed muscle fibres were detected in the area of injury after

intravenous injection of stem cells (Fig. 16). The newly developed muscle fibres still had sarcoplasm of heterogeneous density.

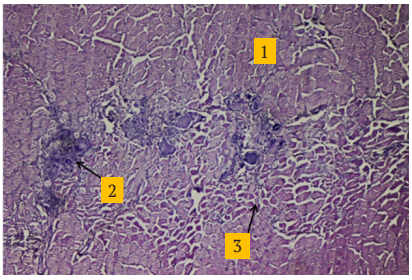

Figure 16. Average part of the experimentally established defect on day 12 after intravenous injection of allogeneic MSCs

Notes: *1 – newly developed muscle tissue; 2 – regeneration focus; 3 – incompletely developed muscle fibres. Karatsi hematoxylin and eosin, ×50*

On the 14th day after the injury, only muscle fibres were detected in the area of injury after intravenous injection of stem cells. However, they still had sarcoplasm of heterogeneous density, and the number of satellite cells on their surface was increased (Fig. 17). Such changes indicated incomplete development of striated muscle fibres in the area of injury. However, between the bundles of muscle fibres, layers of fibrous connective tissue typical for skeletal muscles, in which blood vessels passed, had already been established.

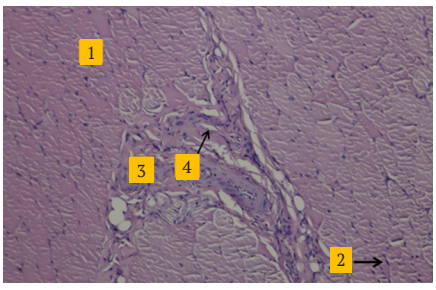

Figure 17. Average part of the experimentally established defect on day 14 after intravenous injection of allogeneic MSCs

Notes: *1 – newly developed muscle fibres; 2 – satellite cells; 3 – fibrous connective tissue layer; 4 – blood vessel. Karatsi hematoxylin and eosin, ×50*

The results of the author's research are compared and analysed with the most recent research conducted by a group of researchers in 2022, which demonstrates the promise of using various methods of treatment applications in veterinary medicine, such as stem cells, in the development, improvement and application in veterinary practice.

In research (Santos *et al.*, 2022), in an experimental model of acute muscle injury, it was suggested that allogeneic MSCs can be used to optimise the muscle healing process through three mechanisms: 1) production of growth factors to promote angiogenesis and prevent cell apoptosis; 2) immunosuppressive effect to reduce the activity of T- and B-lymphocytes; and 3) inducing fibroblasts to differentiate into myocytes. The authors of the publication chose acute muscle injury with early evaluation as stem cells demonstrate greater functionality and productivity in the first days after administration, which is consistent with the results of the evaluation presented in the described work.

Thus, the researchers hope to determine the possible acceleration of functional recovery after using allogeneic MSCs. An important originality of the research is that it is the first study to explore using allogeneic MSCs to treat acute muscle injury in an experimental model. This work is a continuation of the study on using allogeneic MSCs for the treatment of acute muscle injury in an experimental model.

Limitations of this research include difficulties in sampling, as this is a groundbreaking study; using an experimental model that does not fully reflect clinical practice, as acute cut muscle injuries are rare; and the lack of additional evaluation by other methods.

The results of this research emphasise the importance and potential of using allogeneic mesenchymal stem cells in the field of veterinary medicine, in particular, in the treatment of acute muscle injury. Analysing the most re-

cent similar studies (El-Husseiny *et al.*, 2022; Grogan *et al.*, 2022; Santos *et al.*, 2022) and based on the above, it becomes obvious that using MSCs in muscle tissue injury is a promising area of modern veterinary medicine. Allogeneic bone marrow-derived mesenchymal stem cells, collected from recipient animals, act as an effective stimulator of femoral muscle myocyte regeneration.

An inflammatory muscle healing process was observed in both study groups, indicating the presence of inflammatory tissue in the sample. However, the results of the quantitative analysis demonstrated that the addition of ADSC caused a decrease in the number of inflammatory cells per field at the 2-week assessment. The quantitative analysis demonstrated a decrease from 164.2 cells per field in the group without ADSC supplementation to 89.62 cells per field in the group with ADSC supplementation, representing a 46% reduction in the number of inflammatory cells after ADSC supplementation. Considering that the control group did not demonstrate signs of inflammation, this quantification was not performed for the control group.

The author's research has established that using allogeneic MSCs in the repair of damaged muscles helps to accelerate the regenerative process by replacing damaged areas with muscle cells. It was confirmed by microscopic analysis of histological specimens, which demonstrate these changes.

Among other things, it was established that the speed and outcome of the recovery of experimentally damaged muscle tissue in rabbits depends on the method of allogeneic MSCs administration. Injections of cells into the muscles directly (intramuscular injections) demonstrated better results than injections through the vein, and complete recovery was observed on day 14 of the experiment. When cells were injected through a vein after muscle tissue damage, only muscle fibres with incomplete maturity were observed in the area of injury, which had an increased number of satellite cells on the surface. It indicates the incomplete process of fibre development of striated muscle tissue in the injured area. However, typical layers of fibrous connective tissue with blood vessels, characteristic of skeletal muscle, were already present between the fibres. The intravenous administration of stem cells led to a significant acceleration of the recovery of damaged muscles, which coincides with the results of other researchers. However, the activity of regenerative processes was lower than in animals of experimental group 1, which were injected with allogeneic MSCs intramuscularly.

The research results are of great practical importance for the further development and implementation of cell therapy in veterinary medicine, in particular for the effective treatment of muscle tissue injuries in animals, particularly in the case of heavy physical exertion on skeletal muscle and when used in sports competitions. These results demonstrate the potential of cell therapy to improve muscle recovery and may serve as a foundation for the further development of new methods of treating muscle injuries in veterinary practice. Using allogeneic MSCs in clinical practice may have broad prospects for the treatment of humans with muscle damage. These studies may contribute to the development of new therapeutic approaches and open up opportunities for rapid and effective muscle recovery after injuries and in the development of myopathies.

Further research and clinical trials may provide new perspectives in the field of regenerative medicine and contribute to the development of new methods of treating muscle injuries in veterinary medicine.

Conclusions

By modelling the experimental injury of the pelvic head of the biceps femoris muscle in

rabbits, research was conducted to determine the effectiveness of mesenchymal stem cells in terms of the intensity of recovery processes with different methods of their administration (at the site of the experimental defect and intravenous administration), and comparison with the conventional method of treatment. Modelling of experimental muscle tissue damage was performed by dissecting the skin and fascia and cutting off the biceps femoris in the area of the midplane of the pelvic head, measuring 1.5x1.5 cm to a depth of 1.5 cm of muscle tissue.

As a result, it was established that using allogeneic mesenchymal stem cells to stimulate the recovery of experimentally damaged muscles significantly accelerated the recovery of the damaged area by filling the defect with full-fledged muscle cells, which was confirmed microscopically using the histological method of examination.

Therewith, it was determined that the rate and order of restoration of experimentally damaged muscle tissue in experimental rabbits using transplanted allogeneic MSCs depended on the method of their administration. In particular, local (intramuscular) injections of allogeneic MSCs demonstrated better results than intravenous injections and were completely completed by day 14 of the experiment. Therewith, in the case of intravenous injection of stem cells after experimental muscle tissue injury, only muscle fibres were detected in the area of injury, which still had sarcoplasm of heterogeneous density and an increased number of satellite cells on their surface, indicating incomplete formation of striated muscle fibres in the area of injury. However, between the muscle fibre bundles, layers of fibrous connective tissue typical of skeletal muscles with blood vessels were already established.

The results of the experimental studies are of great practical importance for the further development and implementation of cell therapy in clinical veterinary practice, including as a highly effective method of treating muscle tissue injuries in animals, particularly in cases of intense physical activity on skeletal muscle and when used in sports competitions.

In the future, it is planned to conduct comparative studies and clinical trials on the effectiveness of animal cell therapy in the case of spontaneous muscle damage to develop appropriate treatment protocols for similar forms of myopathy and their widespread further implementation in applied veterinary medicine.

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None.

Conflict of Interest

None.

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Мікроскопічні зміни в експериментально ушкоджених м'язах кролів за впливу трансплантованих мезенхімальних стовбурових клітин

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Анотація. Актуальність роботи пов'язана із можливістю штучно коригувати інтенсивність регенерації скелетної м'язової тканини у тварин, що на перспективу істотно вплине на тактику відновлювального лікування м'язових травм. Мета наукового дослідження полягала у визначенні ефективності впливу трансплантованих мезенхімальних стовбурових клітин на відновлювальні процеси в експериментально ушкоджених м'язах кролів. У роботі використано метод гістологічного дослідження мікроскопічних змін в експериментально ушкодженій м'язовій тканині тазової голівки двоголового м'яза стегна кролів. Завдяки мікроскопічним дослідженням процесу регенерації експериментально ушкодженої посмугованої м'язової тканини встановлено, що у тварин після внутрішньом'язового введення алогенних мезенхімальних стовбурових клітин вже на 2 добу досліду навколо створеного дефекту формувалася сполучна тканина. На 4 добу в місці пошкодження м'язової тканини виявляли велику кількість стовбурових клітин у сполучній тканині. На 6 добу в цьому місці відзначали лише відносно невеликі вогнища регенерації м'язової тканини. На 8 добу в цій локації виявлялися лише невеликі вогнищеві скупчення стовбурових клітин. На 10 добу після внутрішньом'язового введення мезенхімальних стовбурових клітин у місці пошкодження з'являлися новоутворені м'язові волокна. На 14 добу після внутрішньом'язового введення мезенхімальних стовбурових клітин у місці пошкодження відзначалися повністю сформовані м'язові волокна та міжм'язова сполучна тканина. У кролів після внутрішньом'язового введення алогенних мезенхімальних стовбурових клітин усі регенераторні процеси проходили активніше за такі у тварин інших дослідних груп і повністю завершувалися на 14 добу досліду. Практичною цінністю отриманих результатів є з'ясування закономірностей розвитку репаративних процесів в ушкодженій ділянці м'яза та визначення ефективності використання трансплантованих алогенних мезенхімальних стовбурових клітин у стимуляції відновлювальних процесів, що важливо враховувати в лікуванні м'язових травм із застосуванням регенеративної терапії

Ключові слова: клітинна інженерія; регенерація; двоголовий м'яз стегна; травма; скелетна мускулатура; гістологічні методи