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Influence of PRP on morphological changes in muscle in the early period after traumatic muscle injury in the experiment

V. Dzhyvak, I. Klishch, T. Datsko, O. Khlibovska

I. Horbachevsky Ternopil National Medical University of the MOH of Ukraine, Ternopil, Ukraine

Dzhyvak V.H. – PhD, postgraduate student of the Department of Pathological Physiology, I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.
ORCID: 0000-0002-4885-7586, e-mail: djyvak@tdmu.edu.ua

Khlibovska O.I. – MD, PhD, Associate Professor of the Obstetrics and Gynaecology Department I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.
ORCID: 0000-0003-3293-0010, e-mail: chlibovska@tdmu.edu.ua

Datsko T.V. MD, PhD, Associate Professor of the Department of Pathologic Anatomy, Autopsy Course and Forensic Pathology I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine. ORCID: 0000-0001-9283-2629, e-mail: datsko_t@tdmu.edu.ua

Klishch I.M. - Prof., DSc, PhD, MD Professor of Department of Functional and Laboratory Diagnostics I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine.
ORCID 0000-0001-6226-4296, e-mail: klishch@tdmu.edu.ua

Abstract. The aim of our study was to investigate the effectiveness of the use of platelet-rich plasma on morphological changes in muscle tissue in traumatic muscle injury in the experiment.

Materials and methods of research. We analyzed the results of histological analysis of the muscle tissue of animals in an experiment in traumatic muscle injury treated with platelet-rich plasma. Depending on the treatment received, the animals were divided into: the first group - with traumatic thigh muscle injury without PRP correction (40 rats), the second

group with traumatic thigh muscle injury with PRP correction (40 rats), the third group - control (12 intact rats). Preparation of PRP took place in two stages.

Results and discussion. As a result of the conducted researches it was established that the introduction of PRP showed differences in morphological features on the seventh day at the beginning of the process of muscle recovery, compared with the first experimental group. There are signs of regeneration, while the muscle fibers are still in the process of formation and without vascularization. This morphological descriptive analysis confirms that in the group, which was treated PRP, was the best organization of the process of restoring muscle.

Conclusions

1. In traumatic muscle injury in experimental animals, structural changes in muscle tissue begin to develop from the first day of the experiment throughout the study period and are manifested by severe circulatory disorders and the development of dystrophic - necrotic changes, especially in epithelial structures, reaching a maximum on the seventh day.

2. The introduction of PRP affects the better organization of the muscle recovery process.

Key words: traumatic muscle injury; PRP; regeneration

Introduction

Multiple and combined traumatic injuries remain an urgent problem of medicine [1]. Mortality from accidents and injuries in Ukraine is growing by an average of 1% annually [2]. According to the WHO, among the causes of death injuries are third, and among the population under 40 - first place.

Muscle injuries are defined as morphological or histochemical changes that cause musculoskeletal dysfunction [8]. Approximately 30% of injuries diagnosed by doctors are related to the muscular system. Muscle injury is one of the most common forms of injury that occurs during sports, causing 10-55% of all injuries [1, 2, 3]. Depending on the severity and location of the injury, different forms of therapeutic treatment are used, from conservative and medical treatment to surgical treatment. Beyond these principles, there is no clear consensus on the treatment of acute muscle injuries. Therefore, the search for effective treatments for muscle injuries is relevant. Platelet-rich blood plasma (PRP) is used to stimulate tissue repair in muscle damage [7, 9].

The use of platelet-riched plasma is a promising area of treatment of diseases of the musculoskeletal system. However, the evidence for its effectiveness is mixed and varies

considerably, depending on the specific indications. Therefore, platelet-rich blood plasma (PRP) is planned to be used to stimulate tissue repair in muscle damage [7].

Platelet-rich plasma is plasma that has a higher than normal platelet concentration and contains PRP growth factors: PDGF - platelet growth factor, TGF-β - transforming growth factor, EGF - epithelial growth factor, VEGF - vascular endothelial growth factor, but also adhesive molecules (fibrin, fibronectin and vitronectin), cytokines. During centrifugation, the resulting plasma contains proteins, fibrinogen, nutrients (glucose, lipids), hormones, vitamins, enzymes, intermediate and final metabolic products, inorganic ions that are involved in the cascade of tissue regeneration [7, 9].

The aim

To investigate the effectiveness of the use of platelet-rich plasma on changes in muscle tissue in traumatic muscle injury in the experiment.

Materials and methods of research. The study was performed on 92 adult nonlinear white rats weighing 180-210 g, which were kept on a standard diet of vivarium. Animals of the experimental group under thiopental-sodium anesthesia (40 mg / kg body weight) using a percussion mechanism simulated injury of the thigh muscle. Depending on the treatment received, the animals were divided into three groups: the first group - with traumatic thigh muscle injury without PRP correction (40 rats), the second group - with traumatic thigh muscle injury with PRP correction (40 rats), the third group - control (12 intact rats).

PRP was obtained by two-stage centrifugation, in the modes: in stage I 1600 rpm, in stage II 2100 rpm antivibration centrifuge LMC-3000 “Biosan” (Latvia), which allowed to maintain the maximum platelet content [7].

Material for histological examination was obtained on the 1st, 7th day after removal of the animals from the experiment by decapitation under thiopental anesthesia. Fragments of the thigh muscles became the material for morphological and morphometric studies. For histological examination, the material (fragments of the thigh muscles) was fixed in a 10% solution of buffered neutral formalin. Further histological preparations were performed according to generally accepted methods [4]. Production of serial paraffin sections with a thickness of 4-6 μm . was performed on a sled microtome. Staining of drugs was performed with hematoxylin and eosin, which studied the structure of normal parenchymal organs, as well as the nature of morphological changes after injury. For photo documentation, images from histological specimens were displayed on a computer monitor using a Nicon eclipse C microscope and a Digital Camera SCMOS using ToupWiev software at various magnifications [4].

The work was performed on the basis of the Department of Pathological Physiology, Pathological Anatomy with a sectional course and forensic medicine of the Central Research Laboratory (certificate of certification № 001/18 dated September 26, 2018) and interdepartmental training and research laboratory (certificate of certification №132 / 17 issued December 29, 2017) I. Horbachevsky Ternopil National Medical University of the MOH of Ukraine.

When working with animals, the rules of handling experimental animals were observed in accordance with the EU Council Directive 2010/63 / EU on compliance with regulations, laws, administrative regulations of the EU Member States on the protection of animals used for scientific purposes [5,6].

Research results and their discussion

Histological examination of muscle tissue on the first day of the post-traumatic period revealed structural changes , manifested by severe circulatory disorders and the subsequent development of dystrophic- necrotic changes, which reached a maximum on the seventh day of the experiment.

On the first day in experimental rats not treated with PRP, found such changes as acute circulatory disorders with focal vascular destruction, destroyed muscle cells impregnated with erythrocytes, edema of the surrounding fibers, tissue detritus, a small number of lympho- and histiocytes, mast cells , single segmental leukocytes (Fig. 1).

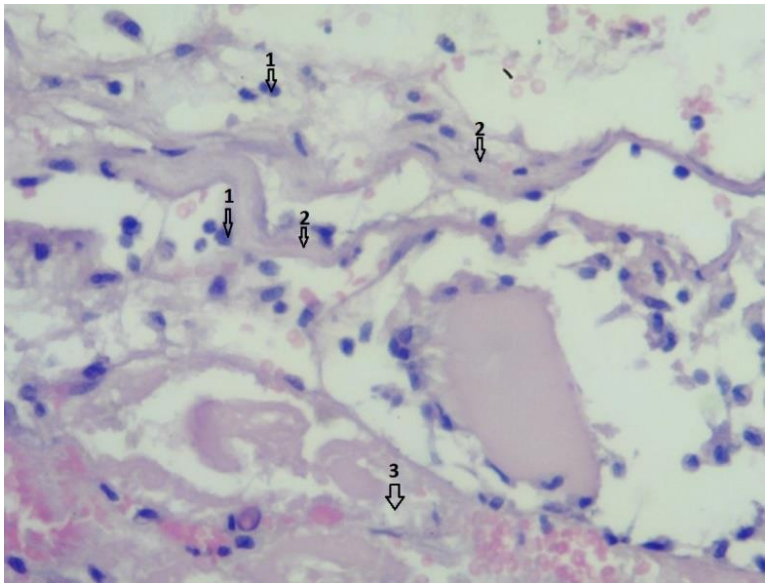

Fig. 1 Muscle area of injury at the 1 day of traumatic muscle injury. 1- moderate leukocyte infiltration, 2- edema of collagen fibers, 3- area of necrotic masses and necrotic changes. Hematoxylin and eosin staining. × 200

In the group of experimental rats injected with PPP on the 1st day in the area of the lesion was found dilation and plethora of blood vessels, an acute inflammatory reaction around the vascular wall, but perivascular edema of the stroma was not visualized. Necrotic changes were observed in the muscles (Fig. 2).

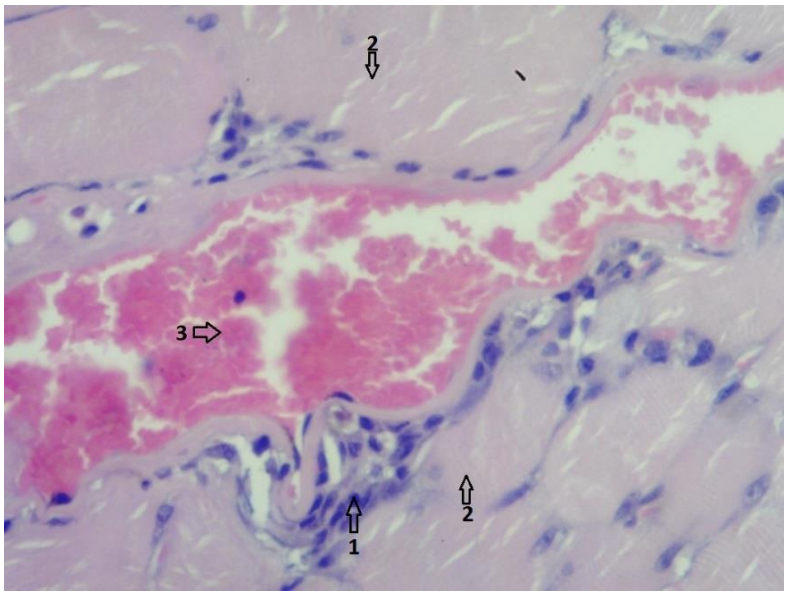

Fig. 2. Muscle structure in the area of injury at the 1 day of traumatic muscle injury when using PRP. 1- inflammatory infiltration, 2-muscle fibers, 3-dilated vessels, plethora. Hematoxylin and eosin staining. × 100.

On the 7th day in the first experimental group in the area of damage were found muscle fibers with areas of destruction in the form of unstructured masses, contractures. The nuclei were poorly visualized or absent. Single fibers of the fibroblastic series and macrophages were found between the fibers (Fig. 3).

However, on the 7th day in the second experimental group with the use of PRP in the damaged area, a significant part of the fibers remained preserved, as evidenced by the presence of nuclei. However, their location was slightly changed. Along with this, moderate dystrophic changes were observed in the studied structures. The fibers are surrounded by a significant part of collagen with signs of edema (Fig. 4).

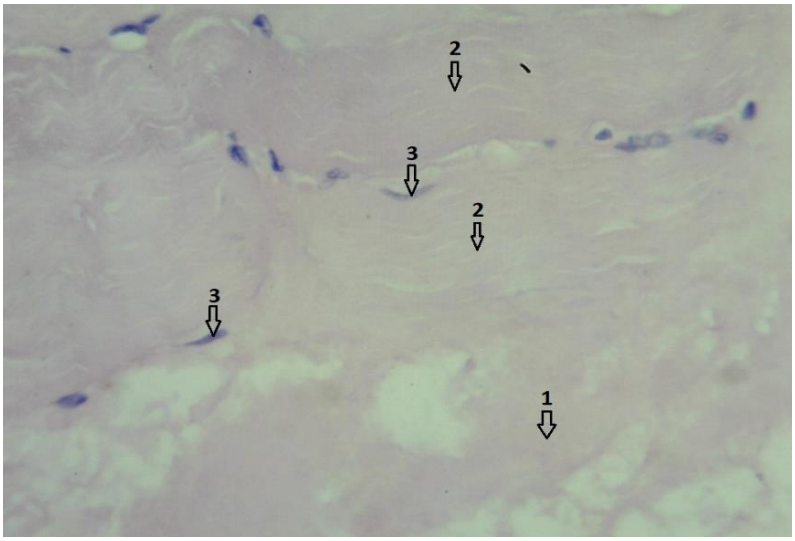

Fig. 3. The muscle structure of the rat in the area of injury on the 7th day of traumatic muscle injury. 1- unstructured masses, probably necrotic changes, 2- muscle fibers, 3- fibroblasts. Hematoxylin and eosin staining. × 200.

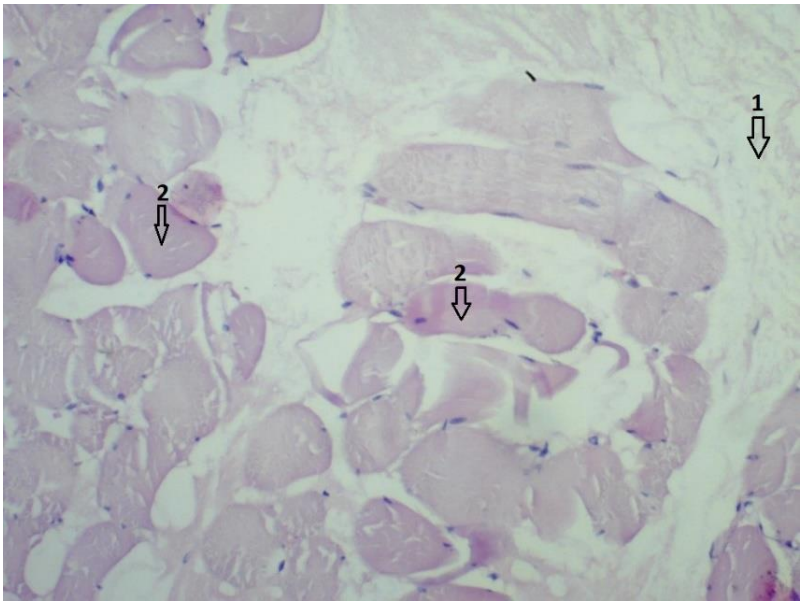

Fig. 4. Muscles of the animal in the area of injury on the 7th day of traumatic muscle injury and PRP correction. 1- collagen fibers with moderate edema, 2- muscle fibers with signs of recovery, improved structure. Hematoxylin and eosin staining. × 200.

The results of histological examination that we received showed us changes in the structural organization of muscle fibers and structural elements of the microcirculatory system. First of all, the violation of the transverse striation of fibers and their morphometric parameters was revealed, which indicates a high level of destruction of contractile elements

and their proteolysis. There is a decrease in the number of nuclei along the structurally altered muscle fibers.

Therefore, it can be assumed that the growth factors contained in PRP play an important role in the regeneration of muscle tissue. The introduction of PRP in traumatic muscle injury has a positive effect on the recovery of muscle fibers, achieving a balance between the lysis of the old cell matrix and the synthesis of a new matrix.

The introduction of PRP showed differences in morphological features on the seventh day of the beginning of the process of muscle recovery, compared with the first experimental group. There are signs of regeneration, while the muscle fibers are still in the process of formation and without vascularization.

This morphological descriptive analysis confirms that the PRP group had a better organization of the muscle repair process. It is possible that the growth factors contained in PRP when injected into the site of injury increase the recruitment, proliferation and differentiation of cells involved in the recovery of muscle tissue and promote accelerated recovery with better tissue differentiation.

Conclusions

1. In traumatic muscle injury in experimental animals, structural changes in muscle tissue begin to develop from the first day of the experiment throughout the study period and are manifested by severe circulatory disorders and the development of dystrophic - necrotic changes, especially in epithelial structures, reaching a maximum on the seventh day.

2. The introduction of PRP affects the better organization of the muscle recovery process.

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