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Full Research Article

RESTORING RATS' ENDURANCE ABILITY AFTER FORCED PHYSICAL EXERCISE UNDER VARIOUS METHODS OF ALLOGENEIC BIOMATERIAL IMPLANTATION

Anna LEBEDEVA[1], Lyalya MUSINA[1], Mars GALAUTDINOV[1], Alexey PRUSAKOV[2], Anatoly YASHIN[2], Vladimir PONAMAREV[2*]

[1] Federal State Budgetary Educational Institution of Higher Education, Bashkir State Medical University, of the Ministry of Health of Russia, 450008, Russia, Republic of Bashkortostan, Ufa
[2] Federal State Budgetary Educational Institution of Higher Education, St. Petersburg State University of Veterinary Medicine, 196084, Russia, St. Petersburg

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Abstract

One of the manifestations of skeletal muscle plasticity is its atrophy, as an adaptive response to catabolic stimuli. They can occur during forced exhausting physical activity. Injection of local anesthetics, glucocorticosteroids, etc., is widely used to correct such pathological manifestations. The purpose of the study was to reveal the skeletal muscle morphofunctional characteristics of experimental animals after forced physical activity under conditions of subcutaneous and combined methods of administration of allogeneic biomaterial (BMA). The model of anaerobic physical exercise was forced swimming of male rats with a load of 10% of body weight. After the swimming test, the animals were divided into four groups. In the first (experimental) group (n=10), the BMA suspension was administered only subcutaneously. In the second (experimental) group (n=10), BMA suspension was injected in combination, i.e., into the muscles of the limbs and subcutaneously. In the control groups, saline solution was administered using similar methods. After tissue collection on days 5 and 21, morphological, physiological, and statistical studies were carried out. The use of BMA contributed to an increase in load tolerance, and accelerated restoration of muscle tissue, hypertrophy, and hyperplasia. There was a decrease in inflammation, and restoration

*Corresponding author – e-mail: psevdopyos@mail.ru

of microcirculation and ultrastructure of muscle fibers: contractile elements, energy balance of cells, and proliferative activation of the nuclear apparatus. Implantation of BMA promoted inhibition of fibrosis, reduction in the number of necrotic muscle fibers, and chemoattraction of macrophages. The greatest effectiveness determined was with the combined administration of the biomaterial. BMA has an actoprotective effect.

Key Words: allogeneic biomaterial, skeletal muscle tissue, fibrosis inhibition, actoprotection

INTRODUCTION

Skeletal muscles exhibit high plasticity under both physiological and pathological conditions. This includes muscle atrophy as an adaptive response to catabolic stimuli. This is facilitated by the exhausting physical activity experienced by professional athletes and individuals engaged in intense professional physical activity (Nemirovskaya and Lomonosov, 2013). In order to correct pathological conditions, injection techniques are widely used, with injections of local anesthetics, glucocorticosteroids, and botulinum toxin type A at the site of injury. The impact on muscles triggers a number of physiological reactions: hormonal – expression of neurosignal substances; cellular – release of biologically active substances, stimulation of metabolism and immunity; capillary – improving blood circulation and rheological properties of blood; reflex – production of signals by skin receptors, their transmission to the corresponding segments of the spinal cord, etc. (Muldashev et al., 2007; Kravtsova et al., 2011).

With local exposure to an allogeneic biomaterial (BMA) for the correction of inflammatory and destructive processes in various tissues and organs, effective healing and inhibition of collagenogenesis were observed (Lebedeva et al., 2023b; Lebedeva et al., 2023c; Muldashev et al., 2011). The development of comprehensive methods of treatment and rehabilitation of people experiencing overtraining is an urgent problem in the light of the selected methods. The purpose of the study was to reveal the morphofunctional characteristics of the hind limb skeletal muscle tissues in experimental animals after forced physical activity under conditions of subcutaneous and combined (i.e., subcutaneous and intramuscular) methods of administering BMA.

MATERIALS AND METHODS

Adult male Wistar rats (200-240 g; n=40) were used in the study. The rats were kept in plastic cages in natural light and a temperature of 22-24ºC, and fed with briquetted feed. Water was supplied without restrictions. All manipulations of the animals were carried out in accordance with the ethical principles approved by the ethical committee at the Federal State Budgetary Educational Institution of Higher Education, Bashkir State Medical University of the Russian Ministry of Health, protocol No. 263 (Ufa, Russia) and established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Directive 2010/63/EU).

The model of anaerobic physical exercise was the method of forced swimming until complete fatigue with a load of 10% of body weight, i.e., the Porsolt test or the despair test, as previously modified (Gostyukhina et al., 2017). Before the experiment, rats were trained for three days to swim without a load for 10 minutes once a day. The swimming test was conducted daily for 30 days. The load weight was adjusted daily according to the weight of the animal.

BMA in dispersed form was used for the study (Vafiev, 2015; Khasanova, 2008). It was made from rat tendons with a particle size of 50-80 μm. The BMA was developed at the Federal State Budgetary Institution, All-Russian Center for Eye and Plastic Surgery of the Ministry of Health of the Russian Federation in Ufa and is manufactured in accordance with Specifications 42-2-537-87.

In group I (experimental) (n=10), a BMA suspension was injected subcutaneously. One bottle (10 mg) was diluted in 5 mL of physiological solution to obtain a 0.2% solution. The method of administration was subcutaneously, once, 0.2 mL in the withers area. In the (control) group (n=10), a similar amount of saline solution was injected into the same body location.

In group II (experimental; n=10), a 0.2% solution of BMA was injected in combination: into the muscles of the fore and hind limbs in a total amount of 4 mL (biceps brachii muscles (*m. biceps brachii*), superficial flexor muscles of the forelimb – ulnar and radial flexors of the wrist (*m. flexor carpi ulnaris*, *m. flexor carpi radialis*) and hind limbs: into the gastrocnemius (*m. triceps surae*), quadriceps muscles of the thigh (*m. quadriceps femoris*) and subcutaneously into the withers area. In the (control) group (n=10) only saline solution was injected into similar areas and in similar quantities.

All injections were performed once. When determining the dose of BMA, results from previously conducted studies with clinical efficacy were taken into account (Lebedeva et al., 2018).

A study was carried out on the maximum duration of swimming before being under water surface for 10 seconds, on days 5 and 21 after the administration of BMA or saline solution. Then, the animals were sacrificed by insufflation of a lethal dose of chloroform vapor.

The muscle tissue of the thigh and calf muscles was excised, fixed in a 10% solution of neutral formaldehyde, dehydrated in a series of alcohols of increasing concentrations and embedded in paraffin according to the generally accepted method. Histological sections were prepared using a LEICA RM 2145 microtome (Germany), which were stained with hematoxylin and eosin according to van Gieson. For immunohistochemical studies, 4 μm-thick paraffin sections were stained using a Leica Microsystems Bond™ immunohistostainer (Germany). MyoD at a dilution of 1:50 (clone 4H207) and CD 68 at a dilution of 1:300 (clone ED1), PCNA, (Santa Cruz Biotechnology, USA) were selected as the primary antibodies. An indirect streptavidin-biotin detection system Leica BOND (Novocastra™, Germany) was used for staining. (Akaji et al., 2022; Brun et al., 2024; Kostilenko et al., 2008).

For electron microscopic examination, tissue pieces were fixed in a 2.5% glutaraldehyde solution prepared in cacodylate buffer (pH 7.2–7.4) with additional fixation in a 1% O_sO_4 solution in the same buffer. The material was dehydrated in alcohols of increasing concentration and poured into Epon-812 according to the generally accepted method. Semi-thin sections 1 μm thick were preliminarily prepared and stained with toluidine blue in a 2.5% anhydrous soda solution. Semi-thin and ultra-thin sections were prepared on an EM UC 7 ultratome (Leica, Germany). Ultrathin sections were contrasted with a 2% aqueous solution of uranyl acetate and Reynolds lead citrate and examined in a JEM–1011 transmission microscope (Jeol, Japan) at an accelerating voltage of 80 kV (Buchholz et al., 2019; Cossio and Hummer, 2018; Xu et al., 2020).

The total number of muscle fibers (MFs), the number of necrotic fibers in the muscle cross-section, and the average cross–sectional area of the MFs were measured. Cells were counted in 20 fields of view of each sample at ×400 magnification. The examination and visualization of the preparations was carried out using a Leica DMD 108 microscope (Germany).

Kruskal-Wallace rank analysis of variance, median (Me) and quartiles (Q1 – 25%; Q3 – 75%) and the Mann-Whitney test to compare the results of individual observation periods within one series of experiments or between them were used. The differences were considered statistically significant at $p<0.05$. Statistical software package Statistica 10.0 was used.

RESULTS

After five days, in control groups I and II, signs of MF destruction, increased variable polygonality, hemorrhages, and perivascular and perimysial edema remained in the muscle tissue of the *m. femoralis* and *m. gastrocremius* (Fig. 1A). MF contractures of the third and fourth degrees were detected, which had led to rupture of the sarcolemma and mosaic necrobiosis. Fragments of damaged fibers were infiltrated with neutrophils and macrophages. The ultrastructural organization of MFs was characterized by loss of transverse striations, disorganization of Z – and M-lines, rupture, and fragmentation of fibers. Mitochondria were in a state of pronounced vacuolization and swelling, with lysis of the cristae. The sarcoplasmic reticulum channels and intermyofibrillar spaces were severely dilated (Fig. 1B). Myelin nerve fibers also underwent pathomorphological changes. They showed swelling, disintegration of the myelin sheaths, and the appearance of vacuoles (Fig. 1C). Areas with intense inflammatory cell infiltration were detected near necrotically damaged MFs (Fig. 1D).

After 21 days, signs of MF destruction remained in the femoral muscles of the control groups: edema, swelling, and inflammatory cell infiltration (Fig. 1E). Collagen fiber deposits were noted in the perivascular and perimysial spaces (Fig. 1F).

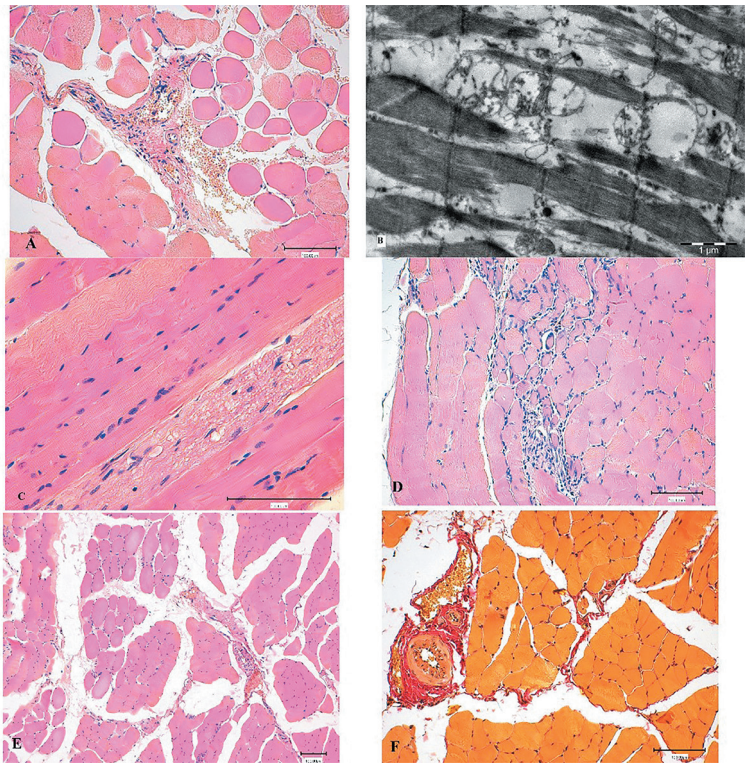

Figure 1. Condition of the muscle tissue of the *m. femoralis* in control groups. **A** – Hemorrhages, edema, polygonal muscle profiles in control group I after 5 days. Hematoxylin and eosin staining. **B** – Disorganization of Z – and M-lines, rupture of muscle fibers, vacuolization of mitochondria and sarcoplasmic reticulum in control group I after 5 days. Electronogram. **C** – Swelling of nerve fibers in control group II after 5 days. Hematoxylin and eosin staining. **D** – Inflammatory cell infiltration in control group II after 5 days. Hematoxylin and eosin staining. **E** – Destruction of muscle fibers of the *m. femoralis* after 21 days. Hematoxylin and eosin staining. **F** – Edema of intrafusal fibers, perivascular and interstitial fibrosis in the *m. femoralis* after 21 days. Van Gieson staining.

Five days after the subcutaneous injection of BMA, signs of both MF damage and the active remodeling of MF were revealed in the femoral muscles tissues. Fiber ruptures, inflammatory cell infiltration, and edema of the perimysial and perivascular space were observed. Overcontraction had led to the loss of transverse striations and swelling of MFs, homogenization of the sarcoplasm, and a change in the MFs' tinctorial properties. However, a high content of muscle nuclei was noted (Fig. 2A). Hemocapillaries were detected with free lumens, without signs of congestion. Endotheliocytes showed signs of active transendothelial metabolism, and pinocytosis was developed (Figure 2B). Accumulations of macrophages, eosinophils, and neutrophils were detected in the perimysium, probably in response to BMA administration (Fig. 2C).

Twenty-one days after the introduction of BMA, inflammatory cell infiltrate, hemorrhages, and increased nuclear activity in MFs remained in the perimysial space of the femoral muscles. As before, contracture changes in the sarcoplasm of grades 1 and 2 were present (Fig. 2D). In the long term, signs of moderate sclerosis of the perimysial and perivascular spaces were found (Figure 2E). Signs of edema, i.e., polymorphic profiles of MF bundles, remained in the calf muscles. An increased number of nuclei in myosymplasts were observed (Fig. 2F).

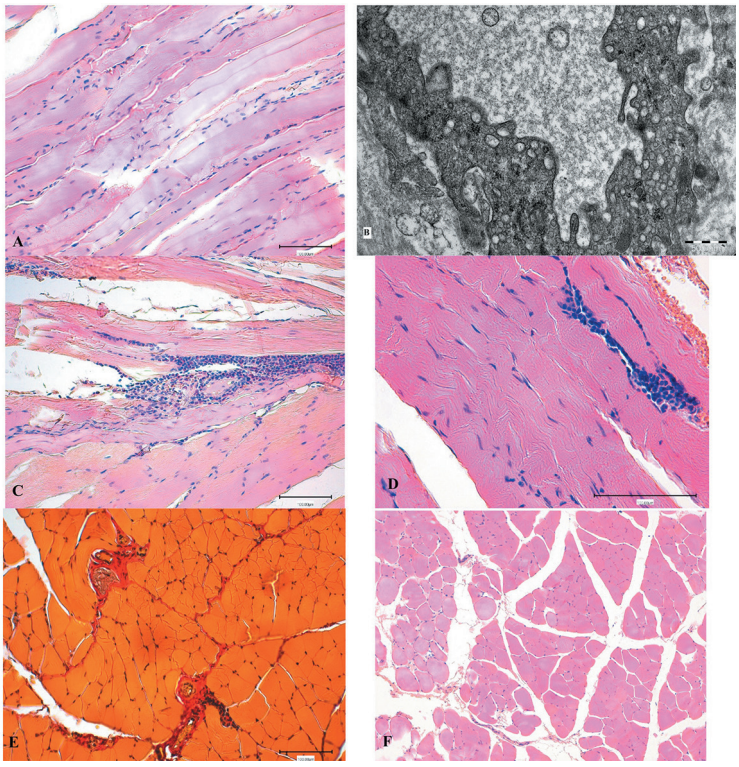

Figure 2. The state of skeletal muscle tissue in experimental group I. **A** – Swelling of muscle fibers, high content of myocytes nuclei in the femoral muscles after 5 days. Hematoxylin and eosin staining. **B** – Active micropinocytosis in hemocapillary endothelial cells after 5 days in the femoral muscles. Electronogram, ×10000. **C** – Inflammatory cell infiltration in the muscle tissue of the thigh muscles after 5 days. Hematoxylin and eosin staining. **D** – Macrophage infiltration in 21 days in the femoral muscles. Hematoxylin and eosin staining. **E** – Perivascular and perimysial fibrosis after 21 days in the femoral muscles. Van Gieson staining. **F** – Rounded muscle fiber profiles of the calf muscle in 21 days. Hematoxylin and eosin staining.

In experimental group II, after 5 and 21 days, the histomorphological picture of the muscle tissue of the femoral and calf muscles did not have any fundamental differences. MF profiles were polygonal, so the fibers fitted tightly to each other. Inflammatory cell infiltration was absent. The morphological structure of the neurovascular bundles was

without any peculiarities (Fig. 3A). There were signs of activation of the mitochondriaome, i.e., clusters of mitochondria with developed parallel oriented lamellar cristae and an increase in the number of intermitochondrial contacts (Fig. 3B). In the early stages, signs of proliferation of progenitor muscle cells and mature fiber nuclei were observed (Fig. 3C). Along with this, high numbers of macrophages were detected in the interfiber space subjected to the indirect immunoperoxidase method for detecting CD 68 (Fig. 3D). There were no signs of increased fibrosis.

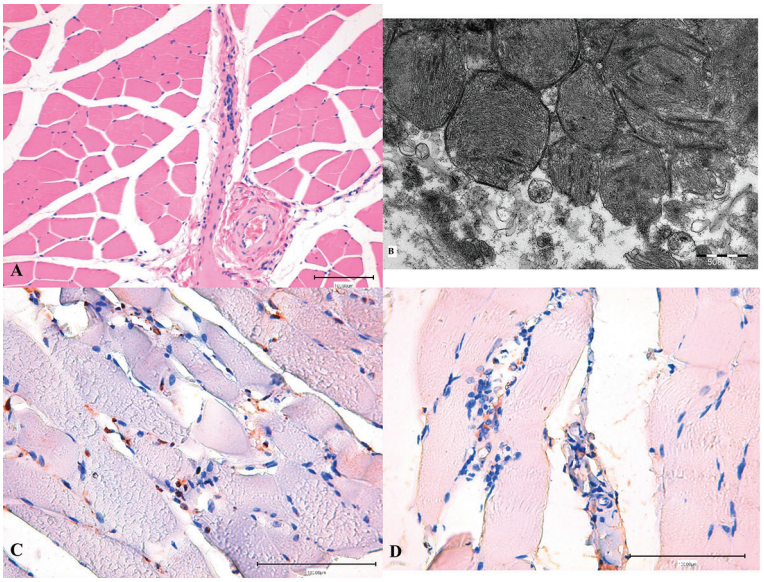

Figure 3. The state of the muscle tissue of the femoral muscles in experimental group II after 5 days. **A** – Polygonality of muscle fiber profiles. Structure of the neurovascular bundle. Hematoxylin and eosin staining. **B** – Restoration of mitochondria from the subsarcolemmal space. Electronogram, ×10000. **C** – Proliferative activity of progenitor muscle cells. Indirect immunoperoxidase method for PCNA detection with hematoxylin staining. **D** – Infiltration of CD 68+ cells in the intermuscular spaces. Indirect immunoperoxidase method for detecting CD 68 with hematoxylin staining.

When comparing the levels of multiplicity of swimming time in rats, it was revealed that between experimental groups I and II, the differences on the days 5 and 21 were statistically insignificant (from $p>0.13$ to $p>0.66$). The swimming time differences between control groups were insignificant (from $p>0.15$ to $p>0.99$). The intergroup differences between each experimental and corresponding control group pairs were significantly higher at both observation periods, with higher parameters in experimental groups.

The average cross-sectional area of the MFs in experimental groups I and II was smaller compared to similar MFs in controls after 21 days ($p>0.12$). The number of necrotic MFs on day 21 after the use of BMA, compared with the control group,

was significantly lower (χ^2=13.7, p<0.002 and χ^2=24, p<0.0001, respectively). The differences between days 5 and 21 after saline administration were significant with subcutaneous and combined administration. When using BMA, the lower number of necrotic MFs on day 21 was significant for both methods of BMA administration (p <0.04 –0.006).

An intergroup comparison of the total number of MFs revealed that with the combined administration of BMA, their number was significantly higher than with subcutaneous administration of BMA after 5 and 21 days. On day 21, with the combined administration of saline, the number of MFs was significantly higher than on day 5 (p<0.0001). However, with subcutaneous saline administration, the differences between MF numbers on both days 5 and 21 were insignificant (p>0.20). When using combined administration of BMA on day 21, the number of MFs was significantly higher than on day 5 (p<0.0001). An intergroup comparison showed that with the combined administration of saline and BMA, the number of MFs in the visual fields differed significantly, with more MFs seen in the experimental group than in the control group (p<0.002). With subcutaneous injection of saline and BMA, the number of MFs was significantly higher in the control group than in the experimental group (p<0.007 – <0.0001), but was significantly lower in the conditions of BMA use (p<0.0001).

Undifferentiated MyoD muscle cells were not identified. The intergroup number of CD 68 cells at the initial time (5 days) was significant (χ^2=29.7, p<0.0001) and was significantly higher in the experimental groups than in both control groups. A significantly higher number of CD 68 cells occurred in the experimental group with combined administration of BMA, and a significantly lower number in the experimental group with subcutaneous administration of BMA (p<0.0005), but more than in similar control groups (p<0.0004; p<0.0005), respectively. After 21 days, CD 68+ cells were detected in single quantities in all experimental groups, and their numbers did not differ significantly between groups (Table 1).

Table 1. Morpho-functional characteristics of skeletal muscles of rats 5 and 21 days after application of allogenic biomaterial (BMA)

Experimental groups	**Days**	**Multiplicity of rat swimming duration (Me (Q1; Q3))**	**Average cross-sectional area of MF (μm^2) ($p<0.05$)**	**Number of necrotic MFs in visual fields (Me; $X_{max} - X_{min}$))**	**Number of MFs in visual fields (Me (Q1; Q3))**	**Number of MyoD cells (in fields of view x200) (Me (Q1; Q3))**	**Number of CD 68 cells (in fields of view x200) (Me (Q1; Q3))**
Subcutaneous injection of BMA	5	1.50 (1.03, 1.56)*	2246.0 ± 432.7	2 (1-3)	37.5 (21, 26)	0	6.25 (5.5; 7) *
	21	2.64 (1.25,4.14)*	2060.7 ± 442.0	1.5 (1-2) *	37 (33, 46.5)	0	1 (1; 3)
Local (intramuscular) and subcutaneous administration of BMA	5	1.78 (1.39, 1.90) *	1868.6 ± 443.3	1 (0-2) *	46 (39, 50.5) **	0	13.75 (12; 14.5) */**
	21	1.60 (1.58, 1.90) *	1360.2 ± 438.0	0 (0-1) *	67.5 (57, 69) */**	0	0,9 (1; 3)
Subcutaneous injection of saline solution	5	0.90 (0.82, 0.98)	1521.0 ± 598.0	3 (1-4)	58 (41.5, 65)	0	3 (2, 4)
	21	1.25 (0.43, 2.10)	2033.2 ± 506.9	2 (2-3)	51 (46, 52)	0	0,8 (1; 2)
Local (intramuscular) and subcutaneous administration of saline solution	5	1.01 (0.88, 1.06)	1881.4 ± 807.2	3 (2-5)	37.5 (32.5, 40.5)	0	1.83 (1; 2)
	21	1.20 (1.15, 1.30)	1518.4 ± 514.1	2 (1-3)	52.5 (40.5, 73)	0	1 (1; 2)

* – significant in comparison with according parameter in control group
** – significant in comparison with according parameter in another experimental group

DISCUSSION

During forced debilitating anaerobic load in control groups I and II at the initial stage, signs of microcirculatory disorders were revealed in muscle tissue: stagnation of blood in the venous bed, hemorrhages, and interstitial edema. Contracture changes in MF III

and IV grades were observed, leading to rupture of the sarcomeres and sarcoplasmic membrane, which caused pronounced inflammatory cell infiltration. This goes along with literature data describing pathophysiological mechanisms during intense physical activity. With a load increase by 10% of the animal's weight, in the blood protein catabolism in the soleus muscle and an increase in lactate levels are observed (Richard et al., 2004). This leads to destruction of the sarcolemma and necrosis of the entire myosymplast. Rupture of the cytolemma leads to a violation of the integrity of the associated cytoskeleton (Karkishchenko et al., 2014). Lactic acid accumulation and acidosis suppress energy production and reduce physical performance. Deep ultrastructural changes in MFs were associated with disturbances in energy metabolism—dilatation of the sarcoplasmic reticulum and T-tubules, destruction of sarcomeres, mitochondria and the mitochondria in general, and depletion of the energy reserves of cells (Lenti et al., 2019; Yao and Tang, 2022). Swelling of intrafusal and extrafusal MFs, as well as nerve fibers, was noted. Perivascular inflammatory cell infiltration was found. Necrotically altered MFs in control groups were gradually replaced by collagen fibers, thereby initiating perivascular and interstitial fibrosis. All this caused a decrease in the level of swimming multiplicity of rats in control groups compared to the corresponding experimental ones.

In experimental group I, after subcutaneous administration of BMA, signs of muscle tissue edema, a high content of muscle cell nuclei, inflammatory cell infiltration, and contracture changes were observed, which were present for up to 21 days. In experimental group II, no pathomorphological signs were detected in the muscle tissue in the initial stages. The restorative effect of skeletal muscle tissue turned out to be more significant. Already in the early stages, signs of cellular and intracellular regeneration, activation, development of chondrioma (development and organization of mitochondria in the cells after its regeneration), restoration of the tubular system, and myofibril ultrastructure were detected. Signs of fibrosis were absent. However, swimming times did not differ statistically significantly between these groups. Consequently, this effect was not cumulative.

Analysis of the tolerant load showed that the swimming time after the use of BMA was higher compared to the control. On comparing the tolerant load and the state of muscle tissue with subcutaneous administration of BMA, it is obvious that pathomorphological changes comparable to the control group remained in muscle tissue, but the swimming time was increased. It can be assumed that the allogeneic biomaterial has not only a local, but also a systemic effect, which requires further confirmation.

The average values of the cross-sectional area of the MFs after both subcutaneous and combined administration of BMA decreased over time, which was likely due to the disappearance of edematous phenomena and the restoration of microcirculation. Also, after the introduction of BMA, the number of necrotic fibers was reduced. This indicated complete muscle recovery and effective elimination of wound detritus. It is known that the products of destroyed cells are antigenic and cause chronic

inflammatory reactions, which can be resolved by increased collagen synthesis (Lebedeva et al., 2019).

Moreover, effective phagocytosis by macrophages that migrate in response to BMA implantation is capable of not only eliminating cellular breakdown products, but also stimulating proliferative effect on myosatellite cells and muscle nuclei (Lebedeva et al., 2023a; Musina et al. 2023). The use of BMA showed an increase in the migratory activity of CD 68 macrophages, but intramuscular application of the BMA turned out to be more effective for the chemotaxis of these macrophages. It was previously noted that biodegradation products of biomaterial stimulate the influx of M1-phenotype macrophages, and induce angiogenesis, chemotaxis of myoprogenitor cells, and their differentiation into muscle cells (Lebedeva et al., 2015). M1 macrophages not only fully utilize wound detritus, but also neutralize profibrogenic growth factors, which results in inhibition of fibrosis. The results of this study confirm previously reported findings. BMA degradation products, including collagen, proteoglycans, glycoproteins, and glycosaminoglycans, attract macrophages to the site of CD 68 implantation, which become regulators of cellular relationships through the production of short – and long-range cytokines (Shangina et al., 2021).

The number of MFs in the visual fields measured after the combined administration of BMA was significantly higher than after subcutaneous administration. The detected proliferative activity of PCNA+ muscle cells, both myosatellitocytes and mature cells in myosymplasts, and the higher number of MFs can serve as evidence of the activation of hypertrophy and hyperplasia (Kitzmann and Fernandez, 2001). Subcutaneous use of BMA did not have this effect. Despite the fact that in the control group (with local and subcutaneous injection of saline solution) there was an increase in the number of MFs in the visual fields over time, still, after the local and subcutaneous use of BMA, the number of visible MFs exceeded the number in the control in the long-term period. MyoD muscle progenitor cells were not detected in the preparations. This could be due to their short life span (up to 2 days), after which they lose the determination factor. The MyoD factor manifests itself during embryonic myogenesis and is at its highest level at the beginning of G1 and at its lowest level during the G1 to S phase transition (Kitzmann and Fernandez, 2001; Battistelli et al., 2022; Rudnicki and Jaenisch, 1995).

CONCLUSION

The use of BMA, both subcutaneously and in combination, contributed to an increase in the tolerant load even in the early stages after BMA administration, accelerating the restoration of muscle tissue, hypertrophy, and hyperplasia, and had an actoprotective effect. The greatest effectiveness was determined with local (intramuscular) administration of BMA. There was a decrease in signs of inflammation and restoration of the microvasculature. The ultrastructure of the MFs was restored: contractile elements, the energy balance of cells, and the

proliferative activation of the nuclear apparatus were enhanced. Intramuscular implantation of BMA promoted inhibition of fibrosis and effective elimination of cellular debris and necrotic MFs due to activation of the macrophage component.

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Authors' contributions

LIA, YVA – experiment modeling, control of the workflow, literature overview; GFM, MAL, PVA – conducted the experiment, animal care, information collection, creating draft of the manuscript; PSV – analyzed results, translated the paper, edited the paper.

Competing interests

The authors declare that they have no competing interests.

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OBNOVLJANJE IZDRŽLJIVOSTI PACOVA NAKON PRISILNOG FIZIČKOG VEŽBANJA POD RAZLIČITIM METODAMA IMPLANTACIJE ALLOGENOG BIOMATERIJALA

Anna LEBEDEVA, Lyalya MUSINA, Mars GALAUTDINOV, Alexey PRUSAKOV, Anatoly YASHIN, Vladimir PONAMAREV.

Kratak sadržaj

Jedna od manifestacija plastičnosti skeletnih mišića je njihova atrofija, kao adaptivni odgovor na kataboličke stimuluse i može se javiti tokom iscrpljujuće fizičke aktivnosti. Aplikacija lokalnih anestetika, glukokortikosteroida, itd., široko se koristi za korekciju takvih patoloških procesa. Cilj studije bio je da otkrije morfofunkcionalne karakteristike skeletnih mišića eksperimentalnih životinja nakon prisilne fizičke aktivnosti pri primeni supkutanog i kombinovanog načina primene alogenog biomaterijala (BMA). Model anaerobnog fizičkog vežbanja bio je prisilno plivanje mužjaka pacova sa opterećenjem od 10% telesne težine. Nakon testa plivanja, životinje su podeljene u četiri grupe. U prvoj (eksperimentalnoj) grupi (n=10), suspenzija BMA je aplikovana samo supkutano. U drugoj (eksperimentalnoj) grupi (n=10), suspenzija BMA je aplikovana kombinovano, tj. u mišiće ekstremiteta i supkutano. U kontrolnim grupama, fiziološki rastvor je aplikovan koristeći slične metode. Nakon uzorkovanja tkiva 5. i 21. dana, sprovedene su morfološke, fiziološke i statističke analize. Upotreba BMA doprinela je povećanju tolerancije na opterećenje i ubrzala obnovu mišićnog tkiva, hipertrofiju i hiperplaziju. Primećeno je smanjenje upale, obnova mikrocirkulacije i ultrastrukture mišićnih vlakana: kontraktilni elementi, energetski balans ćelija i proliferativna aktivacija jedarnog aparata. Implantacija BMA stimulisala je inhibiciju fibroze, smanjenje broja nekrotičnih mišićnih vlakana i hemoatraktaciju makrofaga. Najveća efikasnost utvrđena je kod kombinovane primene biomaterijala. BMA ima aktoprotektivni efekat.

Ključne reči: allogen materijal, skeletno mišićno tkivo, inhibicija fibroze, aktoprotekcija