

STUDY OF BONE MARROW ASPIRATE INJECTION EFFECT ON DENERVATED MUSCLE ACCORDING TO ELECTROMYOGRAPHY STUDIES

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Abstract

The aim. To study in experiment the effect of bone marrow aspirate injection on the course of denervation-reinnervation processes in skeletal muscle using neurophysiological research method.

Materials and methods. Experimental study was performed on 36 mature rabbits weighing 3–4 kg. Experimental model of the peripheral nerve acute injury and neurorrhaphy is taken as a basis. Animals were divided into 4 groups of 9 animals per group (3 animals for each experimental period). At 8, 12, and 16 weeks after surgery, all rabbits underwent needle EMG of the target muscles (m. gastrocnemius, and m. tibialis anterior) to study denervation-reinnervation changes.

Results. Results of target muscles electromyographic examination are shown. As norm, we took motor unit potentials parameters, which were registered during the study of contralateral (not operated) pelvic limbs.

Largest number of registered MUPs at different stages of the denervation-reinnervation process was observed, and was significantly higher ($\alpha=0.07$) in the groups in which bone marrow aspirate was injected in relation to group without bone marrow aspirate injection. Changes in the parameters of the registered MUPs in all groups corresponded to the general characteristics of the denervation-reinnervation process.

Conclusions. Injection of bone marrow aspirate into the target muscles during surgery and in the early stages of reinnervation (in experimental study it is 7 weeks after surgery) – reliably ($\alpha=0.07$) promotes improvement of reinnervation processes in muscles, which is manifested by registration of more motor unit potentials.

Keywords: bone marrow aspirate, denervated muscle, denervation-reinnervation process, electromyography, experimental study.

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1. Introduction

In general structure of injuries – 1.3–2.8 % are traumatic lesions of peripheral nerves of the extremities [1]. Swedish Hospital Discharge Register (1998–2006) reports that peripheral nerve injury occurred at a rate of 13.9 per 100,000 patients per year [2]. In structure of peripheral nerves injuries of the extremities – 77 % belong to the upper extremity [3].

Microsurgical suture and nerve grafting – remain the «gold standard» in the repair of damaged peripheral nerves [4]. S. Mackinnon and A. Dellon, summarizing 40 years of experience, showed that satisfactory recovery results were achieved in only 20–40 % of patients [5]. Ovais et al. analyzing long-term results (27 months) of 75 cases of acute peripheral nerves microsurgical restoration indicate that: excellent results were achieved in 50 % of patients, good – 37.5 %, and

satisfactory – 12.5 % [6]. A. Ruijs in 2005 conducted a meta-analysis on the results of microsurgical reconstruction of peripheral nerves, and showed that satisfactory results of functional recovery were achieved in only 51.6 % of patients [7].

In modern understanding of the denervation-reinnervation process occurring in the system of «peripheral nerve – skeletal muscle» – main reasons for unsatisfactory results are the problem of denervation changes of Schwann cells in distal nerve segment, and neuromuscular synapses [8]. That is proved by works of many authors such as S. Jonsson et al. [8], H. Wu [9], R. J. Balice-Gordon [10], Y. Sugiura [11], W. Liu [12]. And secondary changes that occur in muscle fibers during a long period of denervation [13].

Hogendoorn et al., studying the effect of bone marrow stromal stem cells on denervated muscle in patients with brachial plexus injuries, came to the following conclusion: bone marrow stromal stem cells are able to differentiate into myocyte satellite cells, which significantly reduces hypotrophy, and can stimulate muscle tissue regeneration [14].

From 2015 to 2019, in the Department of «Microsurgery and reconstructive surgery of upper extremity» of the SI «ITO NAMS of Ukraine», in 19 volunteers with neglected brachial plexus and peripheral nerves traumatic injuries, surgery on the nerves were supplemented by injection of concentrated bone marrow aspirate. It was found that in these patients (during examinations every 2–3 months after surgery), electromyography data during reinnervation period of target muscles slightly differed from patients who were not administered bone marrow aspirate, by greater number of motor unit potentials (MUPs) and their parameters. In our opinion, this could be explained in some way by the possibility of bone marrow stromal stem cells differentiation into neurolemocytes (terminal Schwann cells) or myocyte satellite cells, possibility of which was previously proved by a number of experiments by various authors [15–18]. In addition, work of Mochizuki et al. indicate that bone marrow stem cells are able to regulate the manifestations of skeletal muscle fibrosis [19].

In order to confirm or deny our working theory, an experimental study was conducted.

The aim of the research was to study in experiment the effect of bone marrow aspirate injection on the course of denervation-reinnervation processes in skeletal muscle using neurophysiological research method.

2. Materials and methods

From 2018 to 2019, in the SI «Institute of traumatology and orthopedics NAMS of Ukraine» experimental study on 36 mature rabbits weighing 3–4 kg was performed. Experimental model of the peripheral nerve acute injury and neurorrhaphy is taken as a basis. Animals were divided into 4 groups of 9 animals per group (3 animals for each experimental period): a group of pseudooperated animals and 3 groups of experimental animals. Pseudooperated animals underwent revision and mobilization of the right sciatic nerve at the level of proximal third of the thigh (**Fig. 1**). Surgical access was sutured.



Fig. 1. Surgical intervention in a group of pseudooperated animals

Experimental animals (group 1) (**Fig. 2**) underwent mobilization and neurotomy of the right sciatic nerve at the level of proximal third of the thigh. Two end-to-end nerve sutures by non-absorbable monofilament surgical suture EHICON PROLENE® 7/0 (Johnson'n'Johnson Init) using

microsurgical techniques was applied. In order to worsen conditions of reinnervation, nerve suture is applied through the entire thickness of sciatic nerve, with significant tension, and without adaptation of nerve endings. Skin was sutured with a continuous suture using a non-absorbable monofilament surgical suture COROLINE® 2/0 (Peters SURGICAL). Operating field was treated three times (1.5 minutes before complete drying) with Sterillium® Classic Pur disinfectant (manufactured by BODE Chemie GmbH, Germany).



Fig. 2. Surgical intervention in 1 group of experimental animals

Animals of group 2 (**Fig. 3**) after performing a nerve suture (in order to worsen conditions of reinnervation, nerve suture was applied through the entire thickness of sciatic nerve, with significant tension, and without adaptation of nerve endings) was injected with purified bone marrow aspirate into target muscles (m. gastrocnemius, and m. tibialis anterior).



Fig. 3. Surgical intervention in group 2 of experimental animals

In group 3 of experimental animals, purified bone marrow aspirate was administered 7 weeks after nerve suturing (nerve suturing was performed through the entire thickness of sciatic nerve, with significant tension, and without adaptation of nerve endings).

Protocol for obtaining bone marrow aspirate. 0.2 ml of 4 % ACD-A solution was collected in a 5 ml syringe. A 1.2 mm diameter needle was used to puncture the skin in the projection of greater trochanter of femoral bone. Subsequently, drilling movements performed drilling of the outer cortical layer of the greater trochanter, and immersion of the needle to the inner cortical layer. Using a 0.9 mm thick conductor, needle is cleaned of bone debris trapped in the middle of the needle. Syringe was attached to the needle and 2 ml of bone marrow aspirate was collected. Needle was removed, puncture site was treated with Sterillium® classic pur disinfectant (manufactured by BODE Chemie GmbH, Germany). Bone marrow aspirate was purified of spongy bone particles using a Tulip® Emulsifier™ subcutaneous fat aspirate filter. A 0.6 mm thick injection needle is connected to a syringe with a purified bone marrow aspirate. Purified bone marrow aspirate was inserted into the target muscles (m. gastrocnemius, and m. tibialis anterior) on the right pelvic limb.

Electroneuromyographic examination was performed on a Viking Quest electromyograph (USA) by needle electromyography (EMG) and external stimulation of active movements in the muscles of the pelvic limbs. At 8, 12, and 16 weeks after surgery, all rabbits underwent needle EMG of the target muscles (m.gastrocnemius, and m.tibialis anterior) to study denervation-reinnervation changes (**Fig. 4**). Needle electromyography was performed in three stages:

- Stage 1 – registration of insertional activity, which mechanically irritate muscle fibers and causes electrical activity due to insertion of needle electrode into the muscle, which ends when the electrode stops moving. Study of insertional activity was not the subject of this work.

- Stage 2 – examination of the muscle at rest. During muscle denervation, spontaneous activity of muscle fibers is recorded in the form of fibrillation potentials and positive sharp waves.

- Stage 3 – examination of the muscle during active contraction (as a reaction to an external stimulus). Potentials generated by motor units were recorded in muscles. Needle electromyography in the target muscles was used to determine the total number of motor unit potentials (MUPs) and their following parameters: duration, shape (polyphase) and amplitude.



Fig. 4. Electroneuromyography of experimental animals

Duration of motor unit potential reflects motor unit territory - number of muscle fibers that were reinnervated by one axon and their spatial distribution in the motor unit, as well as synchrony of muscle fibers activation.

MUPs amplitude – depends on the number, diameter and density of reinnervated muscle fibers distribution in relation to recording electrode.

Shape (number of phases) of motor unit potential – reflects synchrony of reinnervated muscle fibers action potentials formation and impulse conduction through nerve and muscle fibers. During reinnervation process, number of polyphase potentials (percentage of polyphase) increases.

Statistical analysis was performed in Microsoft Excel software Microsoft Office Professional Plus 2016 package. Following methods of descriptive statistics were used: measures of central trend (average value), range, positive and negative error. Significance of difference between groups was determined using the Mann-Whitney U-test. Results are presented in the form of tables.

Study was approved by the Commission on Bioethics of the SI «Institute of Traumatology and Orthopedics of the National Academy of Medical Sciences of Ukraine», protocol No. 1, dated 19.06.2020. Members of the commission agreed that the materials covered in the article were obtained during the study in compliance with bioethical requirements in accordance with Helsinki Convention of the Council of Europe on Human Rights and Biomedicine and relevant laws of Ukraine.

Experimental manipulations were performed in accordance with the «Regulations on use of animal biomedical research», «European Convention for the protection of vertebrate animals used for experimental and other scientific purposes», and «Guide for the Care and Use of Laboratory Animals».

4. Results

Results of experimental animals target muscles electromyographic examination are shown in Table 1. As norm, we took MUPs parameters, which were registered during the study of contralateral (not operated) pelvic limbs.

Table 1

Results of experimental animals EMG examination

Term	Group	Total number of MUPs:	Total number of polyphasic MUPs:	Average duration of all MUPs: (ms)	Average amplitude of all MUPs: (μV)
8 weeks	Norm	37±2.56	8±0.94	15.8±3.46	4382±2568.63
	1 group	8±0.94	5±0.90	8.4±8.93	223±297.16
	2 group	7±1.67	7±1.67	5.9±8.36	116±164.59
	3 group	15±1.26	8±1.60	9.1±7.42	294±329.24
12 weeks	Pseudo.	53±3.08	22±1.37	14.6±3.60	2756±1262.38
	1 group	28±3.30	15±1.26	14.7±2.21	1439±1026.23
	2 group	43±1.46	18±1.29	16.6±2.97	864±143.52
	3 group	46±3.90	13±1.67	13.2±2.48	1372±602.49
16 weeks	Pseudo.	46±1.70	13±1.46	15.3±2.67	3218±1458.79
	1 group	38±1.25	11±0.69	13.5±1.89	1307±606.50
	2 group	44±1.60	15±2.57	13.2±2.21	917±275.07
	3 group	51±2.50	12±1.29	12.1±1.52	1099±400.19
	Pseudo.	34±1.80	7±0.90	18.1±2.80	4526±2086.60

5. Discussion

During the second stage (muscle at rest) of electromyographic study, we determined spontaneous activity of target muscles muscle fibers. Severity of spontaneous denervation activity observed in target muscles of experimental groups (except for pseudooperated) did not differ significantly between the groups.

In our study, we also noted changes in motor unit potential parameters in the target muscles of pseudooperated animals, which in our opinion can be explained by the asynchrony of nerve impulse conduction by axons, which could occur due to the small number of axon loss during nerve mobilization, or development its local ischemia due to mesoneurium fibrosis. Within 16 weeks after surgery, we recorded parameters that approached the parameters of non-operated animals.

At 8 weeks after neurotomy and neurorrhaphy in the muscles of all animals of experimental groups (groups 1–3) was a low number of motor unit potentials, which is explained by early stage of reinnervation processes in target muscles. Within 12 weeks after surgery, there was a significant increase in the number of motor unit potentials, which continued to increase for 16 weeks.

The largest number of registered MUPs at different stages of the denervation-reinnervation process was observed, and was significantly higher ($\alpha=0.07$) in the groups in which bone marrow aspirate was injected (group 2 – where bone marrow aspirate was injected during surgery, and group 3 – in which injection of bone marrow aspirate performed at 7 weeks after surgery) in relation to group 1. Given the number of registered motor unit potentials, following can be noted:

- Group 1 (where only neurotomy and sciatic nerve neurorrhaphy were performed without injection of bone marrow aspirate) was characterized by a significantly lower number of motor unit potentials ($\alpha=0.07$) with a gradual tendency to increase their number, but the amount of MUPs in all study periods was the lowest.

- Group 2 (in which bone marrow aspirate was injected during surgery) was characterized, compared to the first group, by a relatively large number of motor unit potentials ($\alpha=0.15$). More-

over, in the first period (8 weeks) the number of MUPs was comparable to group 1, but starting from the second period (12 weeks) exceeded the parameter of 1 group.

– Group 3 (where bone marrow aspirate was administered 7 weeks after surgery) was determined to have significantly the highest number of motor unit potentials ($\alpha=0.06$) at all stages of the study. Within 8 weeks after surgery, the number of MUPs was almost twice as high as in groups 1 and 2, but from 12 weeks the difference in the amount of MUPs between groups 2 and 3 did not differ significantly.

Changes in the parameters of registered MUPs in all groups at different times after surgery were correlated with corresponding stages of denervation-reinnervation process. We observed an increase in the duration, amplitude and polyphase of motor unit potentials between 8 and 16 weeks.

Our study may in some way correlate with the study of Hogendoorn et al. [14], but in their work they did not study ENMG characteristics of denervation-reinnervation process. Also, our study confirms the results obtained by Farjah et al. [20] of improvement of target muscles reinnervation in terms of their stimulation by bone marrow aspirate.

6. Study limitations

The limitations of our study were impossibility of continuous monitoring of the denervation-reinnervation process dynamics. Because the design of our study involved the withdrawal of experimental animals at 8, 12 and 16 weeks after surgery, we did not monitor EMG dynamics in the muscles of individual animals at the stages of denervation-reinnervation process, but only recorded changes in MUPs in individual groups. Due to this, we could not track true dynamics of changes in parameters of registered motor unit potentials, and therefore draw conclusions about the trends of their change in different groups.

7. Prospects for further research

In our opinion, study involving monitoring of denervation-reinnervation process continuity may be promising. And also research which would consider parameters of each separate MUPs and target muscles mapping.

8. Conclusions

1. Injection of bone marrow aspirate into the target muscles during surgery and in the early stages of reinnervation (in experimental study it is 7 weeks after surgery) – reliably ($\alpha=0.07$) promotes improvement of reinnervation processes in muscles, which is manifested by registration of more motor unit potentials.

2. Injection of bone marrow aspirate into the target muscles did not significantly affect the parameters (duration, amplitude and shape) of registered motor unit potentials, changes in all groups correlated with corresponding stages of denervation-reinnervation process, and morpho-functional adjustment of motor units.

3. Problem of the influence of bone marrow aspirate on denervation-reinnervation processes occurring in skeletal muscles needs further in-depth study.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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