UDC 616.833.58-018-089.84-085.849.19-003.93-07-092.9
DOI: 10.14739/2310-1237.2023.3.282793

Influence of shortwave laser therapy on morphometric parameters of sciatic nerve regeneration after experimental neurorrhaphy

Z. M. Yashchyshyn[1,A,B,C], O. O. Dyadyk*[2,D,E,F]

[1]Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine, [2]Shupyk National Healthcare University of Ukraine, Kyiv

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

The aim of this work was to reveal the effectiveness of delayed laser therapy for the regeneration of the sciatic nerve.

Materials and methods. In 21 rats, transection and epineural microsurgical suture of the right sciatic nerve (SN) were performed. In 16 rats (experimental group) in the period from 1.0 to 2.5 months after surgery, a course of laser therapy (LT-1, n = 8) and a delayed (after 6 months) course of laser therapy (LT-2, n = 8) were used 18 sessions were performed in each subgroup. Laser therapy was not performed in 5 operated animals and they were included in the control group. The terms of withdrawal of rats from the experiment were 2.5 and 12.0 months after the operation. During the morphometry of semi-thin sections distal to the level of the sutures, the volumetric density of the constituent elements of the SN, the number density of myelinated nerve fibers (MNF), their diameters, the thickness of the axons and myelin sheath, the myelination index (index G) distribution of MNF by diameter and dependence of index G on the fiber caliber were measured. For double control, we used muscle morphometry data from intact rats (Intact). Muscle tissue was additionally examined by the method of transmission electron microscopy using a generally accepted technique with a REM-100 electron microscope (Selmi, Sumy, Ukraine).

Results. In the control group, as compared to the intact group, the numerical density of muscle fibers was 21 % higher, but 38 % less than the numerical density of microvessels, therefore, the vascularization index was reduced by 1.7 times. The obtained results indicated laser compensation for denervation atrophy, which was confirmed by morphometric data.

Conclusions. The results of the study indicate a stimulating prolonged effect of delayed laser therapy in regenerative axonogenesis.

Key words: sciatic nerve, regeneration, morphometry, laser therapy.

Pathologia, 2023. 20(3), 261-265

***E-mail:** alena0566@gmail.com

Вплив коротхвильової лазерної терапії на морфометричні показники регенерації сідничного нерва після експериментальної нейрорафії

З. М. Ящишин, О. О. Дядик

Мета роботи – оцінювання ефективності відстроченої лазеротерапії для регенерації сідничного нерва (СН).

Матеріали та методи. У 21 щура здійснили перетин правого сідничного нерва і наклали епіневральний мікрохірургічний шов. У 16 щурів (експериментальна група) у період від 1,0 до 2,5 місяця після операції застосували курс лазеротерапії (ЛТ-1, n = 8) і відтермінований (через 6 місяців) курс лазеротерапії (ЛТ-2, n = 8); у кожній підгрупі здійснили 18 сеансів. У 5 прооперованих тварин лазерну терапію не виконували, і вони залучені в контрольну групу. Терміни виведення щурів з експерименту – 2,5 і 12,0 місяця після операції. Під час морфометрії напівтонких зрізів дистальніше рівня швів встановили об'ємну щільність складових елементів СН, щільність мієлінових нервових волокон (МНВ), їхній діаметр, товщину аксонів і мієлінової оболонки, індекс мієлінізації (індекс G), визначили розподіл МНВ за діаметром і залежність індексу G від калібру волокна. Для подвійного контролю результатів здійснили морфометрію нервів в інтактних щурів. М'язову тканину додатково дослідили методом трансмісійної електронної мікроскопії, застосувавши відому методику, електронний мікроскоп РЕМ-100 (фірма «Selmi», м. Суми, Україна).

Результати. У контрольній групі порівняно з інтактною кількісна щільність м'язових волокон на 21 % більша, але на 38 % менша від кількісної щільності мікросудин; отже, індекс васкуляризації знижений в 1,7 раза. Результати, що одержали, свідчать про лазерну компенсацію денерваційної атрофії; це підтверджено морфометричними даними.

Висновки. Результати дослідження підтвердили стимулювальний пролонгований ефект відстроченої лазерної терапії при регенеративному аксоногенезі.

Ключові слова: сідничний нерв, регенерація, морфометрія, лазерна терапія.

Патологія. 2023. Т. 20, № 3(59). С. 261-265

According to A. Eberstein & S. Eberstein [1], the results of studies over the past 25 years have proven that the biochemical and physiological properties of muscle fibers are determined not so much by the influence of neurotrophic substances as by the activity of the muscle; therefore, different types of myostimulation are considered the most effective way to treat denervation atrophy.

Schimrigk K. et al. found that electrical stimulation not only slows down the development of atrophy of the denervated muscle, but also increases the number of necrotic muscle fibers and reduces the number of myotubes [2,3].

Laser therapy (LT) does not change the number of fibers in the denervated muscle, and the ratio of the proportions of muscle tissue, blood vessels and connective tissue is "improved" in comparison with unstimulated control. The effect of LT on reinnervation has also been the subject of research. Nakano S., Bianciardi G. et al., who found that when low-intensity LT was used to stimulate nerve regeneration after its compression, the cross-sectional area of muscle fibers in the reinnervated muscles increased, and the proportion of connective tissue in them decreased [4,5].

After transection and suture of motor nerves, some authors obtained data on the improvement of the structure and function of reinnervated muscles under the influence of electrical stimulation, others concluded that reinnervation was suppressed [6].

Aim

The aim of this work was to reveal the effectiveness of delayed laser therapy for the regeneration of the sciatic nerve.

Materials and methods

The experiments were carried out on 21 adult outbred rats. In 21 rats under intravenous barbituric anesthesia and aseptic conditions, complete transection and primary microsurgical suture of the sciatic nerve (SN) were performed at the level of the middle third of the thigh. We used the Aesculap company's tools, 8/0 gauge thread on atraumatic needles from Ethicon and 8× magnification of an operating microscope from Opton.

In the postoperative period, in 5 animals, no effects on the regenerative process were applied (unstimulated control – CG). In the experimental group (EG), low-intensity LT courses were conducted on the "Impulse-CT" apparatus with a wavelength of 480 nm of the blue spectrum. Each 10-minute procedure consisted of two parts: the first part (5 minutes) – short-wave laser stimulation of peripheral segments, the second part (20 minutes) – short-wave LT of the nerve trunk proximal to the level of damage. For shortwave LT [8] sciatic nerve, the SN trunk in the middle third of the thigh was irradiated. The procedures were performed every other day (Monday, Wednesday, Friday). The radiation mode is variable, the frequency is 50 Hz, the pulse duration is 0.7 ms, the pulse duration is 2 s; pulse-pause ratio 2:2. The strength of the pulsed irradiation was selected individually. In 16 rats (experimental group) in the period from 1.0 to 2.5 months after surgery, a course of laser therapy (LT-1, n = 8) and a delayed (after 6 months) course of laser therapy (LT-2, n = 8) were used 18 sessions were performed in each subgroup. Laser therapy was not performed in 5 operated animals and they were included in the control group (CG, n = 5). The terms of withdrawal of rats from the experiment are 2.5 and 12.0 months after the operation.

12 months after the operation, the animals EG and CG were withdrawn from the experiment by the administration of a lethal dose of barbiturates. Since the timing of the appearance of the first electro-physiological signs of the reinnervation of the leg muscles in CG and EG rats varied individually from 3 to 8 weeks after the operation, additional 3 experiments were performed using the "prevented regeneration" model (Denerv) [8]: after resection of 3 cm SN, its proximal and distal stumps were isolated with caps made of biologically inert material. Denerv group withdrawn from experience 33 days after the surgery. That made it possible to obtain homogeneous initial data on the structure of the denervated muscle in the period immediately preceding the reinnervation and the beginning of laser therapy sessions. Maintenance, feeding of animals, operations and LT sessions, as well as withdrawal from the experiment were carried out according to the protocol "On humane behavior with laboratory animals" No. 775.

After euthanasia for morphological studies, fragments of the tibialis anterior muscle were taken from the local region at the level of its upper, middle and lower third. Transverse cryostat sections were made (microtome-cryostat M05050) to detect the activity of myosin-ATPase according to Padycula, Herman in the modification of A. E. Meijer [9] which allows you to identify different types of muscle fibers in muscles with different compositions. The preparations were digitized on the APK "DiaMorph" and morphometric using the Image program. For double control, we used muscle morphometry data from intact rats (Intact).

Muscle tissue was additionally examined by the method of transmission electron microscopy using a generally accepted technique with a REM-100 electron microscope (Selmi, Sumy, Ukraine).

Statistical processing of the primary data was carried out using the software capabilities of MS Excel-2000, the variational series were ranged with a step of 10.0 μm, and histograms of the distribution of muscle fibers by diameter were obtained. The vascularization index (VI) is the ratio of the number of hemocapillaries to the total number of muscle bundles that make up this muscle, i. e. it reflects the "saturation" of the object with vessels.

Results

Visual analysis of preparations of the denervated muscle indicates significant fiber atrophy as early as 1 month after resection of the SN. The distribution curve by fiber diameters is significantly shifted to the left compared to the intact one and has a high peak instead of a plateau (*Fig. 1*).

In all experiments with a nerve suture one year after the operation, the shapes and sizes of the profiles of muscle fibers in cryostat sections indicate laser compensation for denervation atrophy, which is confirmed by morphometric data. In the control group, the average curve of the distribution of muscle fibers by diameter practically coincides with the intact one (*Fig. 1*). In the LT-1 subgroup, it is shifted to the right and has a small peak. In the LT-2 subgroup, the shift of the curve to the right is even more pronounced, the height of its peak decreases, and the width of the base increases significantly. The study of individual histograms of animals of this group showed that in one rat out of three, the distribution of muscle fibers in diameter bimodal, which, presumably, can be associated with the unequal effect of electrical stimulation on muscle fibers of different metabolic types.

According to the stereological analysis, in the control, compared to the intact muscle, the volumetric density of microvessels is 1.5 times reduced, the proportion of endomysium is 1.2 times reduced and, accordingly, the volume density of muscle fibers is slightly increased (*Fig. 2*). In the LT-1 subgroup, the volumetric density of muscle fibers is comparable to that of intact muscle, but

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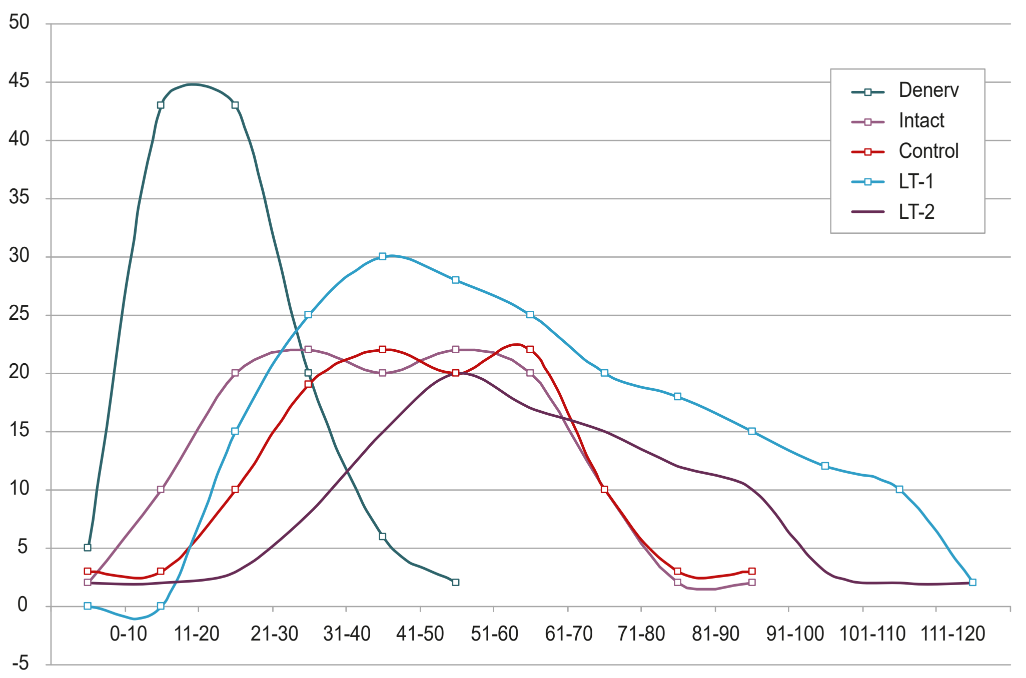

Fig. 1. Curves of distribution of fibers of the tibialis anterior muscle of rats by diameter in an intact state and different experimental conditions. The abscissa axis is the size class of fibers (0–10 microns; 11–20 microns, etc.); Y-axis – percentage of fibers of each class.

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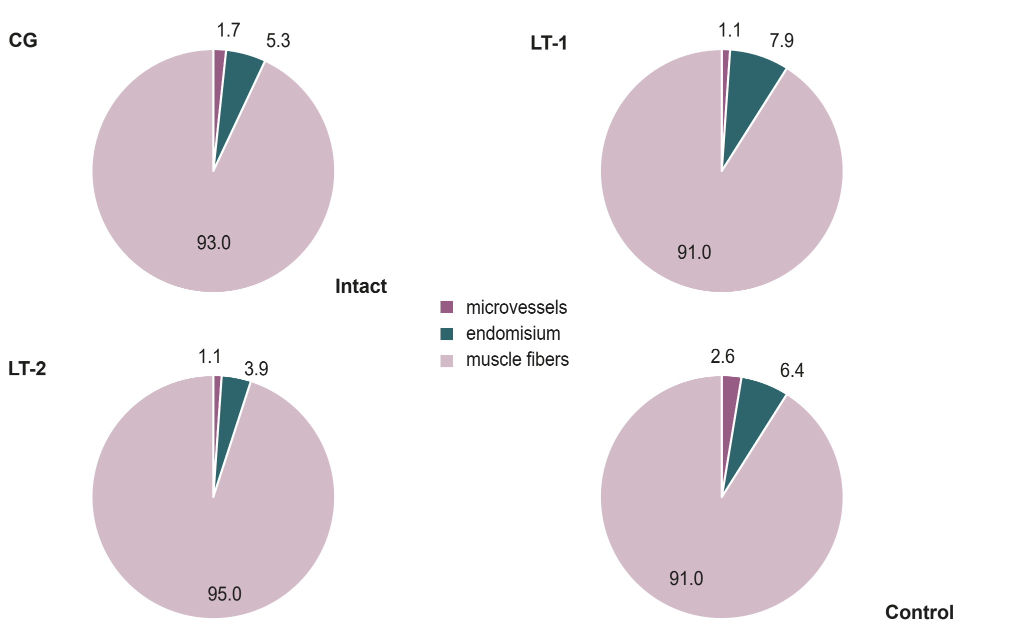


Fig. 2. Volume ratios of muscle fibers, endomysium and microvessels in the tibialis anterior muscle of operated and intact rats.

the volumetric density of microvessels is reduced by 2.4 times, and the proportion of endomysium is increased by 1.2 times. In the LT-2 subgroup, the volumetric density of microvessels is less than in the control, but higher than in the LT-1 subgroup; the volume fraction of endomysium in this group is the smallest; even in comparison with an intact muscle, it is reduced by 1.7 times; the bulk density of muscle fibers slightly exceeds the corresponding parameter of other groups.

In the control group, as compared to the intact group, the numerical density of muscle fibers is 21 % higher, but 38 % less than the numerical density of microvessels, therefore the vascularization index is reduced by 1.7 times (*Fig. 3*). In the LT-1 subgroup, the numerical density of muscle fibers is practically equal to the corresponding parameter of the intact muscle, but the numerical density of microvessels is reduced by 1.9 times and the vascularization index is reduced by 2 times. In the LT-2 subgroup, the numerical density of muscle fibers is 1.3 times less than in the intact muscle, the numerical density of microvessels is 1.8 times, the vascularization index is reduced 1.4 times.

Despite this, an increase in ATPase activity was observed in the group with LT-1 (*Fig. 4*), which is closely correlated with the expansion and normalization of the lumen of hemocapillaries (*Fig. 5*).

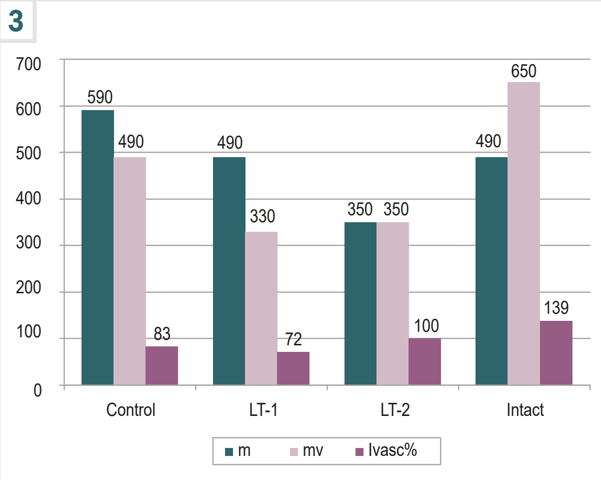
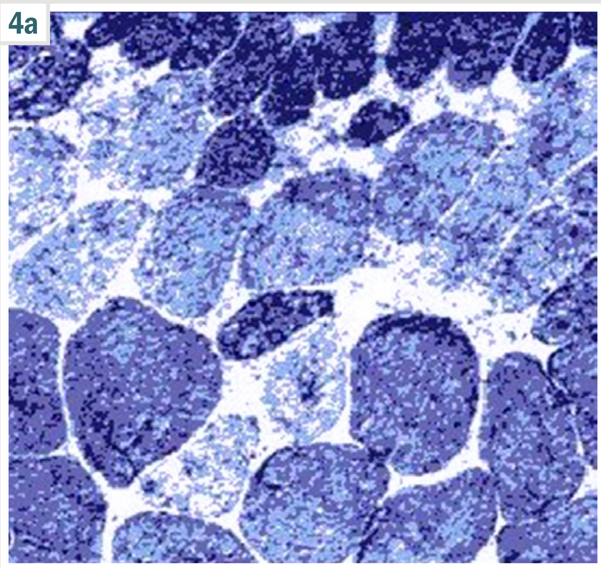
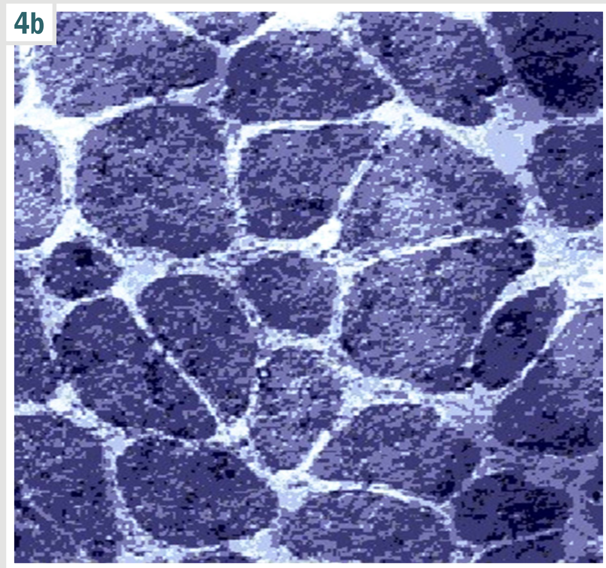


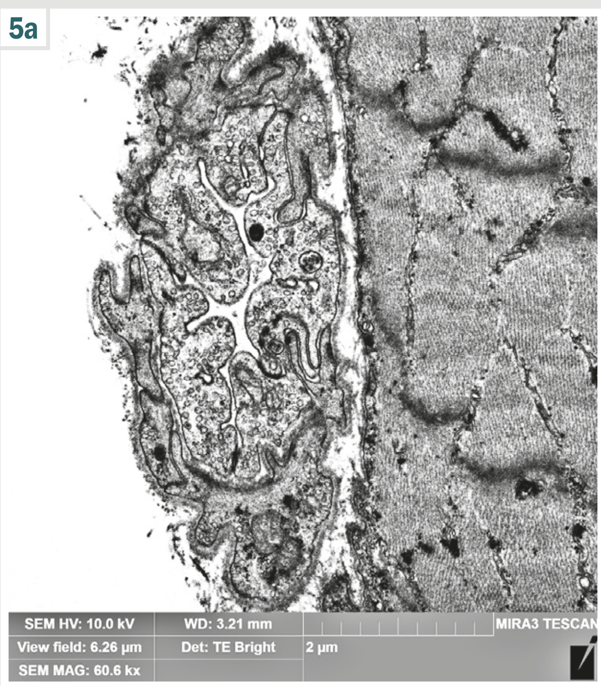
Fig. 3. Numerical density of muscle fibers (**mf**), microvessels (**mv**) and vascularization index (**Ivasc%**) in the tibialis anterior muscle of operated and intact rats after laser therapy.

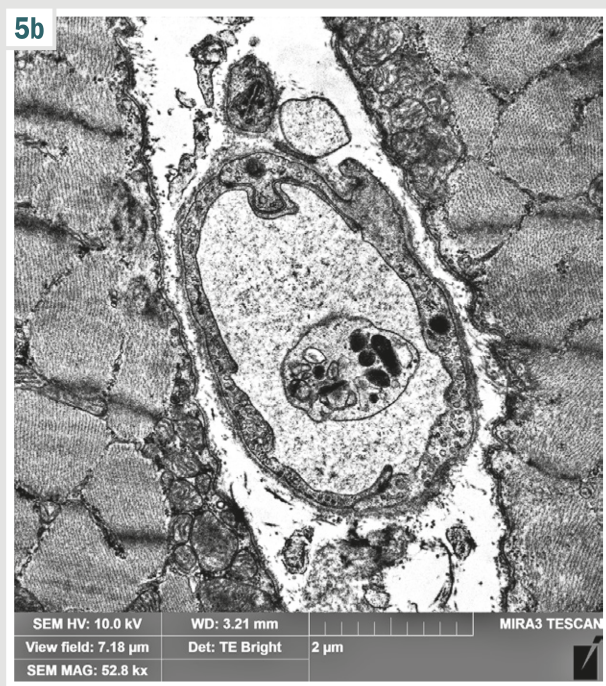
Fig. 4. ATPase activity in muscle fibers m. tibialis anterior before (**a**) and after (**b**) laser therapy in conditions of SN neurorrhaphy.

Fig. 5. Structural organization of hemocapillaries SN before (**a**) and after (**b**) laser therapy in conditions of SN neurorrhaphy. Method: transmission electron microscopy. Magn.: ×10 000.









Discussion

The results of our study indicate that in rats after 1 month of denervation, pronounced atrophy of the anterior tibial muscle occurs. In the process of reinnervation, the size of the muscle fibers is restored even without the use of LT, but the tissue organization of the muscle 12 months after the transection and suture of the nerve is not optimal, which is confirmed by the data of other authors [5,6]. First, compared with intact muscle, the volume fraction of microvessels, their number per unit of muscle cross-sectional area and the average number per muscle fiber remain reduced [7]. Secondly, the increased volume and numerical density of muscle fibers indicate an increase in their number as a result of regenerative myogenesis, which often leads to the formation of abnormally branching muscle fibers or small clusters, which does not contribute to muscle restitution [10].

In the group with one course of LT, its effect on the thickness of muscle fibers was established – judging by the nature of the distribution curve, on average they become larger than in the control and in the intact muscle. The volumetric and numerical density of muscle fibers is comparable to that of an intact muscle, but all vascularization parameters are reduced not only in comparison with the intact muscle, but even in comparison with the control. Apparently, LT, carried out in the period from 1.0 to 2.5 months after the operation, not only maintains normo- and induces hypertrophy of muscle fibers experiencing denervation, but also has an inhibitory effect not only on regenerative myogenesis, but also on angiogenesis.

Conducting the second course of LT within 6.0 to 7.5 months after the nerve suture enhances the hypertrophy of the reinnervated muscle. The vascularization index increases due to a decrease in the number density of muscle fibers, the volume and number of density of microvessels remain reduced compared with the controls and especially with intact muscle.

Conclusions

1. The protocol of laser therapy tested in our experiments has a distinct modulating effect on the tissue organization of the reinnervated muscle, contributing to the development of muscle fiber hypertrophy. This effect is accompanied by an increase in muscle vascularization compared to unstimulated controls.

2. An increase in muscle vascularization is accompanied by an increase in the ATPase activity of muscle fibers and indicates a positive regenerative potential of laser therapy.

3. An increase in the ATPase activity of muscle fibers along with an increase in their vascularization makes it possible to use these indicators in assessing the effectiveness of the means that contribute to the recovery of muscle function after experimental neurorrhaphy of the sciatic nerve under the conditions of laser therapy.

Prospects for further research are to determine correlational relationships between the processes of regeneration of nerve fibers of the sciatic nerve and the degree of vascularization during laser therapy at different times after applying a microsuture.

Funding

The work was carried out within the framework of a comprehensive scientific research topic: "Pathogenetic mechanisms in development of changes in organs of the respiratory, endocrine, nervous systems in the modeled pathological states and their correction", state registration number 0117U001758 (2017–2022).

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Надійшла до редакції / Received: 31.05.2023
Після доопрацювання / Revised: 21.08.2023
Схвалено до друку / Accepted: 14.09.2023

Information about authors:

Yashchyshyn Z. M., MD, PhD, Head of the Department of Physical Therapy, Occupational Therapy, Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine.
ORCID ID: 0000-0001-8672-1797
Dyadyk O. O., MD, PhD, DSc, Professor, Head of Department of Pathological Anatomy and Forensic Medicine, Shupyk National Healthcare University of Ukraine, Kyiv.
ORCID ID: 0000-0002-9912-4286

Відомості про авторів:

Ящишин З. М., канд. мед. наук, доцент, зав. каф. фізичної терапії, ерготерапії, Прикарпатський національний університет імені Василя Стефаника, м. Івано-Франківськ, Україна.
Дядик О. О., д-р мед. наук, професор, зав. каф. патологічної анатомії та судової медицини, Національний університет охорони здоров'я України імені П. Л. Шупика, м. Київ.

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