MORPHOLOGICAL CHANGES IN THE SCIATIC NERVE IN EXPERIMENTAL MODELING OF CONTUSION INJURY OF THE SPINAL CORD IN RATS

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Objective. To analyze morphological and morphometric changes in the sciatic nerve of rats after the spinal cord injury.
Material and Methods. The T9 moderately severe contusion injury of the spinal cord was simulated in 12 Wistar female rats. Functions of the pelvic limbs were assessed according to the standardized BBB scale. The animals were withdrawn from the experiment after nine and 13 weeks. Epoxy semi-thin (1 μm) sections were used to study sciatic nerve at the light-optical level.
Results. Significant recovery of pelvic limb functions was observed within four weeks after surgery, the plateau was achieved by Week 5 (9.5 ± 0.28 points according to the BBB scale), the deterioration in the motor activity was observed by Week 9 (8.67 ± 0.33), its recovery was achieved by Week 13 of the experiment (9.5 ± 0.87). After 9 and 13 weeks, reactive-destructive changes were detected in the sciatic nerve in 9 % and 8 % of nerve conductors, an increase in the number density of myelin fibers by 28 % and 27 % ($p < 0.05$) and myelin-free fibers by 20 % and 49 % ($p < 0.05$), and a decrease in axon diameters by 8 % and 10 % ($p < 0.05$), respectively.
Conclusions. The morphological and morphometric changes in the sciatic nerve revealed after the spinal cord injury in the form of destruction of a part of the fibers, axonal atrophy and a decrease in the proportion of large fibers negatively affect its conductive properties. The leveling of peripheral nerve damage, possibly, will accelerate the regression of the motor deficit caused by the spinal cord injury; therefore, it is necessary to develop a set of preventive measures aimed at preventing the reorganization of the peripheral nerve tissue.
Key Words: rat, contusion injury of the spinal cord, functional assessment using BBB scale, sciatic nerve, morphology, morphometry.
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The spinal cord injury has severe physical and social consequences. It is still a serious and extremely difficult problem of modern medicine [1–3].

The morphological changes of the spinal cord at the injury level have been extensively described in the literature. It has been reported that a primary mechanical abnormality of cells causes a secondary pathway, resulting in their progressive death [4]. The pathological process involves initially intact areas of the spinal cord, and the expansion of the destruction zone happens both in the cranial and caudal directions [5, 6], followed by remodeling [7–9] of the lesion and the formation of cyst cavities and/or glial cicatrix. The injured area significantly exceeds the size of the primary necrotic lesion by the time the destructive processes are completed [10].

The disorder of CSF and blood circulation at the level of injury, as well as the formation of connective tissue and glial structures result in the pathological processes outside the spinal cord. Therefore, during the first month after the injury, a reactive-degenerative and dystrophic restructuring happens in the nervous apparatus of the sympathetic ganglions [11, 12]. Meanwhile, there is a decrease in the morphometric parameters of neuromuscular junctions. In the early post-traumatic period, the muscular system remains morphologically intact. Nevertheless, in the future, the absence of motor functions and neurotrophic disorders cause functional hypotrophy and, as a consequence, the atrophy development [13, 14].

Therefore, after the spinal cord injury, degenerative changes in the nervous tissue are more caudal than the level of damage throughout its entire length, starting from the injury lesion and ending with the neuromuscular junction. Meanwhile, only a few papers contain information concerning changes in the morphology of the tibial nerves in spinal cord injuries at the level of the thoracolumbar junction [15]. The data concerning changes in sciatic nerves in the late post-injury period, on which the morphofunctional state of the extremity depends, have not been found in the relevant literature. Nevertheless, there are sufficiently precise descriptions of changes in the sciatic nerve during its injury.

The objective is to analyze morphological and morphometric changes in the sciatic nerve of rats after contusion injury of the spinal cord in an experiment.

Design: an experimental study.

Material and Methods

An experiment model. Twelve female Wistar rats aged 9–12 months, with a body weight of 270–320 g (experimental series) underwent laminectomy at the T9 level under general anesthesia (Rometar 2 %: 1–2 mg/kg; Zoletil 100: 10–15 mg/kg). A spinal cord contusion was carried out according to the modified Allen technique [16] using an original impact device. The free fall of a load weighing 10 g from a height of 25 mm guaranteed

the development of replicable neurological and anatomical changes corresponding to spinal cord injury of moderate severity [17, 18]. Following the injury, a subdural hematoma was observed; the dura mater preserved its integrity. The surgical wound was sutured layer-by-layer in a complete closure. The laminectomy level was evaluated by a control abdominal X-ray performed in a lateral projection. An additional warming of the animals was done in the early postoperative period. Moreover, acute posttraumatic complications were treated [19–21]. We have applied the maintenance and care of animals in accordance with GOST 33216-2014.

The study was approved by the Ethics Committee of the Academician G.A. Ilizarov National Medical Research Center of Traumatology and Orthopedics (Protocol No. 2 (57) dated May 17, 2018). It was performed in compliance with the principles of animal welfare in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. We also applied Directive 2010/63/EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes.

Methods and criteria of behavioral study. An open field test of rats, based on the analysis of pelvic limb movements, was done in the morning before feeding. In order to properly assess the testing sessions, video monitoring was carried out. In order to standardize and generalize behavioral studies, BBB scale was used from 0 (absence of spontaneous motor activity) to 21 (normal coordinated movements with side-by-side paw placement) [22]. According to findings, a modified score card was filled in and the key of the score decryption of the score card data was used [23]. The animals were withdrawn from the experiment after 9 and 13 weeks. The control group consisted of 10 intact rats.

Histological techniques. The sciatic nerves of the right and left extremities of rats were dissected after 9 and 13 weeks of the experiment, fixed and put in epoxy resin. A polychrome technique (methylene blue, azur II and diamond fuchsine) was applied to stain the semi-thin sections of the nerves. The morphometric studies performed in digital images of semi-thin sections obtained by AxioScope A1 microscope with AxioCam camera. "Video Test Master-Morphology 4.0" was used to determine the diameters of myelin fibers, axon diameters, thickness of the myelin layer, number densities of myelin and myelin-free nerve fibers in 1 mm^2 of the bundle area, the percentage of fibers with signs of reactive-destructive changes. The histograms of the myelin fiber distribution by diameter (step – 1 microns) were designed. Control group: sciatic nerves of 10 intact rats.

Statistical analysis. The statistical significance of the differences between the experimental and control groups was identified by the Wilcoxon criterion for independent samples using the "Attestat" software (version 9.3.1, Rospatent certificate No. 2002611109; developer: I.P. Gaidyshev). Tabular data: average and standard error of the mean (M ± m).

Results

A behavioral assessment. All the animals showed 0 points of neurological impairment on the 1st day after surgery. The first signs of self-reduction of motor activity of the pelvic extremities were recorded no earlier than 3 days after the surgery. A significant recovery of pelvic limb functions was observed within 4 weeks after surgery, reaching a maximum by the 5th week (9.50 ± 0.28 points), after which the recovery curve reached a plateau. The achieved level of recovery remained stable until the 9th week. Then there was a worsening of motor activity (8.67 ± 0.33), after which the achieved level was restored again by the 13th week of the experiment (9.50 ± 0.87).

At the end of the experiment, the nature of the movements of the pelvic limbs of animals varied from support on a limb with a load only in the phase of standing (immobility) to rare steps with support on the sole, provided that the body weight is being maintained.

A histological evaluation. The epineurium, perineurium and endoneurium of the sciatic nerves of the rats of the experimental group remained intact after 9 and 13 weeks. The epineurium, in comparison with the control, contained an increased number of perivascular mast cells, fibroblasts and fibrocytes. Plasma cells and macrophages were found. The arteries and veins of the epineurium had dilated lumen filled with blood corpuscles. Subperineural edema was found in a part of fascicules of a particularly small diameter.

After 9 and 13 weeks, morphological examination of nerve conductors in the nerves of rats of the experimental group showed that some of them were reactively and destructively changed. The deformation or atrophy of their axons, swelling and vacuolization, stratification of the myelin layer, and Wallerian degeneration were observed (Fig. 1). After 9 weeks, the percentage of changed conductors exceeded ($p < 0.05$) the norm by 2.5 times; after 13 weeks – by 2.2 times (Table 1). The numerical ratings of the density of myelin and myelin-free fibers exceeded the norm after 9 weeks by 28 and 20 %, respectively ($p < 0.05$), and after 13 weeks - by 27 and 49 %, respectively ($p < 0.05$).

The study of the population structure of myelin nerve fibers demonstrated that their average diameters after 9 and 13 weeks were reduced ($p < 0.05$) by 7 % (Table. 2). The diameters of axons during these periods were decreased by 8 and 10 %, respectively ($p < 0.05$). The thickness of myelin and the values of the G coefficient did not vary from the control values.

The research of the distribution of myelin nerve fibers by diameters demonstrated that the base of the histograms of the experimental nerve after 9 and 13 weeks of the experiment was shortened by 2 digits on the right (Fig. 2). There were no conductors with a diameter of more than 12 microns (in the nerves of rats of the control group, the fibers varied in diameters from 1 to 14 microns). After 9 and 13 weeks, the histograms of nerves in the rats of the experimental group became unimodal. The

only mode was in the range of 6.1–7.0 microns (Fig. 2b, c), while the histogram of the nerves of the controlled rats had a bimodal pattern: the first mode was in the range of 3.1-4.0 microns, the second – 7.1–8.0 microns (Fig. 2a). The share of small conductors (D ⩽ 4.0 microns) in the experimental group during the studied periods was comparable to normal values (in the control group – 22 %, in the experimental group – 21 %); the share of medium (D – 4.1–7.0 microns) raised by 15% (in the control group – 31 %, in the experimental group – 46 %), and the share of large (D > 7 microns) reduced by 14 % (in the control group – 47 %, in the experimental group – 33 %). The share of the most wired myelin fibers (D > 10 microns) in the studied periods decreased to 6 % (in the control group – 10 %).

Discussion

It is known that peripheral nerve injury triggers a series of retrograde and anterograde reactions [24] characterized by transganglionic degeneration and glial reactions in the affected segments of the spinal cord, as well as the processes of reorganization of nervous tissue in the distal part of the stump [25]. The retrograde changes of the nervous system evolve naturally. They are interrelated with peripheral degenerative processes (Wallerian degeneration) [26]. In turn, the motor neuronal death in the lesion with local spinal cord injury and functional depression in structurally normal organs and systems remote from the lesion [27] cause destructive processes in peripheral nerves.

Although the sciatic nerve is responsible for a significant part of the innervation of the pelvic extremities, there is no enough data on its condition in the pathology of the spinal cord. Therefore, while modeling subarachnoid hemorrhage at the L1 level, the pathological changes in the sciatic nerve are observed already on the 14th day. The number density of degenerated axons increases, and their swelling is registered [28]. If a contusion injury of the spinal cord is simulated at the lumbosacral level (T13/L1), a significant decrease in the diameter of the axons (by 21–31 %) and the thickness of the myelin of nerve fibers (by 20–28 %) of the tibial and common peroneal nerves is observed on day 42. The statistically significant differences in the total number of myelinated axons per unit area were not identified, which is explained by their slow degeneration. Nevertheless, there is very little data on the degeneration rate of peripheral nerves after spinal cord injury [15].

We have found similar, but less pronounced changes in the dimensional characteristics of nerve conductors in the sciatic nerve 9 and 13 weeks after modeling contusion injury of the spinal cord at the T9 level. Their average diameters declined by 7 %, axon diameters – by 8–10 %, but the myelin thickness did not change. The histograms of the distribution of myelin fibers by diameter changed from a bimodal to a unimodal pattern. The anterograde reactive-destructive changes found in 8–9 % of nerve conductors with the preservation of nerve tunics match neuropraxia and axonotmesis [29].

Nevertheless, unlike the study by Wen et al. [15] of tibial nerves on 42nd day after spinal cord injury, we found an increase in the number density of myelin and non-myelin fibers of the sciatic nerve per unit area (from 20 to 49 %). This is a consequence of sprouting, the replacement of one large myelinated fiber by a bundle of small ones following Wallerian degeneration, the formation of new Axon-Schwann cell interactions as a phenomenon of post-traumatic regeneration [30, 31].

Conclusion

The morphological and morphometric changes in the sciatic nerve revealed after the spinal cord injury in the form of destruction of a part of the fibers, axonal atrophy and a decrease in the proportion of large fibers negatively affect its

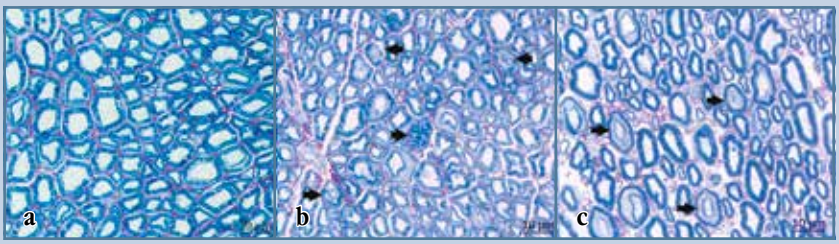

Fig. 1
The fragments of transverse semi-thin sections of the sciatic nerve of rats from the control **(a)** and experimental groups after 9 **(b)** and 13 **(c)** weeks of the experiment; arrows – reactive-destructive changes in fibers; staining: methylene blue, azur II and diamond fuchsine; len.100; oc. 10

Table 1
Trends in the indicators of numerical density of myelin (NA_{mf}), myelin-free (NA_{amf}) nerve fibers and the proportion (D %) of destructively abnormal wires

Group / experiment duration	D %	NA_{mf}	NA_{amf}
Control	3.69 ± 0.46	20466 ± 430	4072 ± 448
Experimental / 9 weeks	9.14 ± 0.99*	26256 ± 76*	4878 ± 788*
Experimental / 13 weeks	8.19 ± 0.01*	26056 ± 1056*	6059 ± 609*

* If $p < 0.05$, then the differences between the experimental and control groups are significant according to the Wilcoxon criterion for independent samples.

Table 2

Change in the average diameters of myelin fibers (D_{mf}), axons (D_{ax}), myelin thickness (L_m) and G coefficient (D_{ax}/D_{mf}) at the experimental stages

Group / experiment duration	D_{mf}	D_{ax}	G	L_m
Control	6.65 ± 0.06	4.70 ± 0.05	0.708 ± 0.002	0.98 ± 0.01
Experimental / 9 weeks	6.18 ± 0.12*	4.33 ± 0.15*	0.704 ± 0.010	0.93 ± 0.02
Experimental / 13 weeks	6.17 ± 0.29*	4.25 ± 0.20*	0.694 ± 0.000	0.96 ± 0.05

* If $p < 0.05$, then the differences between the experimental and control groups are significant according to the Wilcoxon criterion for independent samples.

conductive properties. The leveling of peripheral nerve damage, possibly, will accelerate the regression of the motor deficit caused by the spinal cord injury. The reactive-destructive changes of the sciatic nerve in spinal cord injury are the consequence of complex neural responses. It is possible to apply them as criteria for the development of activities and options for therapeutic strategies aimed at preventing the transformation

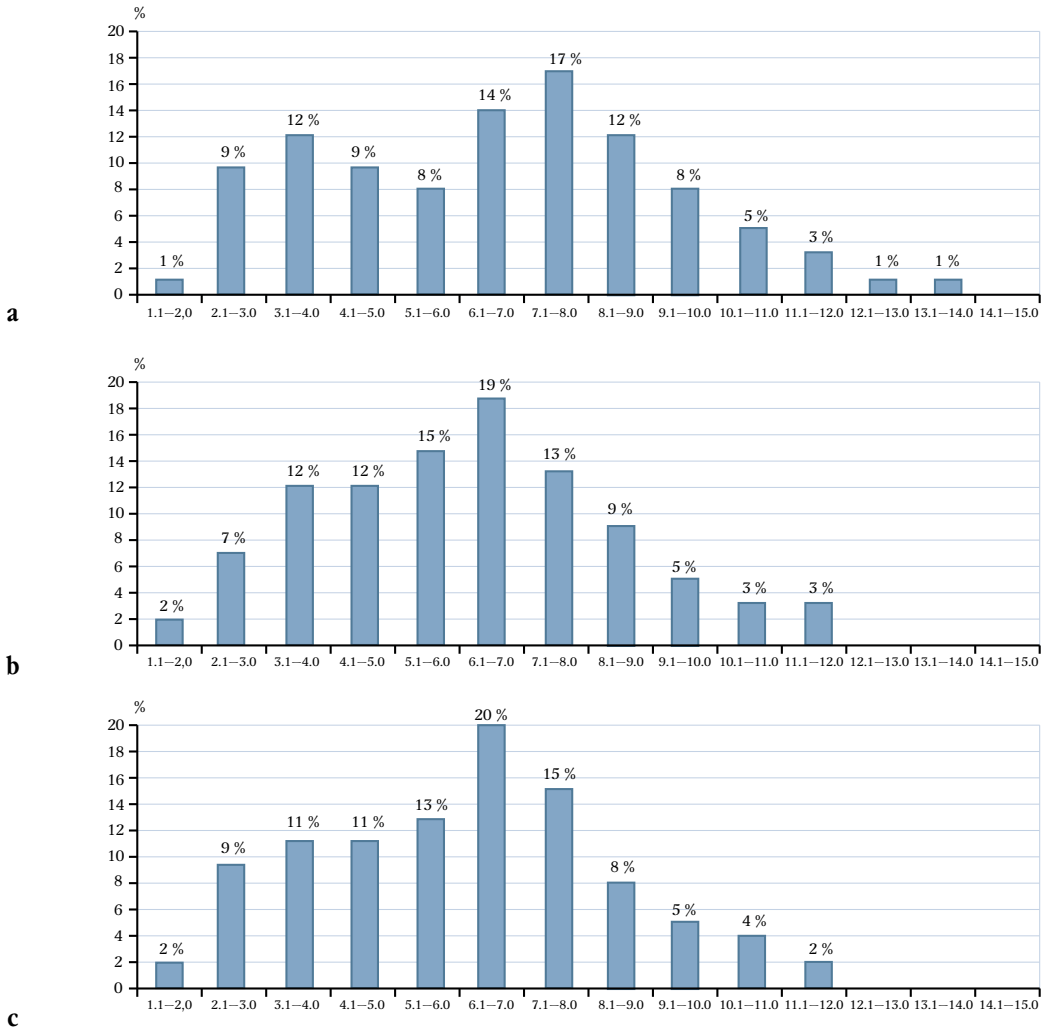

Fig. 2

The histograms of the diameter distribution of myelin nerve fibers: **a** – sciatic nerves of the rats from a control group; **b** – of the rats from an experimental group after 9 weeks of the experiment; **c** – of the rats from an experimental group after 13 weeks of the experiment; X-axis – dimensional classes of fibers, Y-axis – percentage shares of each fibers' class

of the nervous tissue of the central and peripheral parts.

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The authors declare that they have no conflict of interest.

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