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HYPERVASCULARISATION OF REGENERATIVE NEUROMA

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Although the post-traumatic morphological changes as well as those taking place in the course of nerve trunks regeneration have been extensively studied, there are few quantitative data on nerve fibers and microvessels of the regenerative neuroma. This study is aimed at obtaining the morphometric data on nerve fibers and capillaries of the regenerative neuroma of the rats' sciatic nerve 3, 6, 12 and 24 weeks after neurotomy. The study has revealed a significant increase in the density of capillaries and nerve fibers in regenerative neuroma at the initial stages of the experiment. By 24 weeks after surgery, these indicators were normalized. It may be concluded that hypervascularisation of the regenerative neuroma of the peripheral nerve after its injury provides for the metabolic and plastic needs of regenerating axons and connective tissue cells.

Key words: regenerative neuroma, sciatic nerve, hypervascular, rats.

Peripheral nerve injuries are known to be an important medical, biological and social problem. Although the post-traumatic morphological changes as well as those taking place in the course of nerve trunks regeneration [8, 9], specific features of these processes caused by the use of stem cells [7, 14] or silicon nanocrystals [10] have been extensively studied, several aspects of posttraumatic reactive changes in the nerves remain understudied. In particular, quantitative data on nerve fibers and microvessels of the regenerative neuroma are limited [2]. At the same time, the outcome of nerve regeneration largely depends on the processes occurring in this part of the damaged nerve.

The purpose of the work was to perform a morphometric study of nerve fibers and capillaries of the regenerative neuroma of the rats' sciatic nerve at different terms after neurotomy.

Materials and methods. Experiments were conducted on 60 white rats weighing 180-230 g. In 40 animals the right sciatic nerve was transected in its midthird under aseptic conditions and hexenalum anesthesia without subsequent suturing. The animals were sacrificed by an overdose of hexenal 3, 6, 12 and 24 weeks after the surgery. The vessels of the lower extremity were injected with Indian ink-gelatine via the abdominal aorta. The nerves at the surgical site were harvested with the adjacent parts of the central and peripheral stumps. The material was fixed in 10% solution of the buffered neutral formalin. In one half of the cases the frozen sections were prepared which were impregnated with silver nitrate. Part of the sections was additionally stained with azur II-eosine. Density of nerve fibers and capillaries was determined according to the technique [1]. In one part of the cases the total nerve preparations were made in which microangioarchitecture was studied. 10 sham operated and 10 intact rats served as controls. Statistical Analysis: The results were expressed as the mean ± standard error of the mean. The data were analyzed using the Origin Lab version 8.0. The Kolmogorov-Smirnov indicated that the data were not normally distributed. Intergroup differences in sample data were evaluated by the nonparametric Kruskal-Wallis test.

Results and their discussion. Three weeks after the surgery the formation of regenerative neuroma was observed between the central and the peripheral stumps. Its connective tissue basis was represented by granulation tissue rich in macrophages, fibroblasts, mast cells, lymphocytes, collagen fibers and microvessels (fig. 1). The latter were chaotically located, passing in different directions (fig. 2). The density of the newly formed capillaries was 124.7±1,5 mm/mm^3 (Table 1). Nerve fibers having the density of 116298±3595 mm/mm^3 and bands of Bungner have penetrated the connective tissue. Nerve fibers were located relatively irregularly (both longitudinally and obliquely or transversely with respect to the longitudinal axis of the nerve), at the ends of many of them growth cones were determined. We have also determined the density of nerve fibers in the central nerve stump, which was 47674±2195 mm/mm^3.

At week six fibroblasts and collagen fibers prevailed at the regenerative neuroma site, indicating the organization of its connective tissue.. Microvessels and nerve fibers, as earlier, were located in a relatively irregularly. The density of nerve fibers has increased significantly (table 1).

At week 12 regenerative neuroma was represented by loose connective tissue containing microvessels and nerve fibers whose density has decreased in comparison with the previous term and did not significantly differ from the values observed three weeks after the surgery. Microvessels and nerve fibers were located in a more regular fashion than earlier. Many of them were oriented longitudinally, with a small part passing obliquely or transversely.

At week 24 the volume of regenerative neuroma significantly diminished. So did the density of microvessls and nerve fibers which it contained and approached the control values. Microvessels and nerve fibers were located in a relatively regular fashion. Most of them were longitudinally oriented, with only an insignificant part passing obliquely or transversely.

Table 1

Density of capillaries and nerve fibers in the sciatic nerves of different groups of rats

Groups	Capillaries density, mm/mm³ M±m	Density of nerve fibers, mm/mm³ (in the upper third) M±m	Density of nerve fibers, mm/mm³ (in the neuroma) M±m
Intact	74.1±0.9	48571±2234	
Sham-operated	75.8±1.2	47928±2165	
3 weeks	124.7±1.5*⁺	47674±2195	116298±3595*⁺
6 weeks	148.6±0.8*⁺&	48139±2201	124640±3982*⁺ &
12 weeks	126.0±1.1*⁺&	48446±2259	115951±3484*⁺ &
24 weeks	77.1±1.9 &	48571±2234	50208±2686&

Notes: *- significant with respect to intact rats ($p<0.05$); + - significant with respect to sham-operated rats ($p<0.05$); & - significant with respect to the previous term value ($p<0.05$)

Thus, already early after the injury, the density of nerve fibers at the site of regenerative neuroma increases significantly. The fact that the number of the newly formed axons at the site of regenerative neuroma is more than two times that in the central stump draws attention. This is consistent with the data [3] showing that the specificity of post-traumatic target organs reinnervation is achieved due to the formation of several axons branching from a single nerve fiber in the central stump. When one of the axons reaches the relevant organ (a muscle, skin, etc.) the others are reduced. In our study, this was observed 12 weeks after the surgery. And at week 24 the density of nerve fibers in the neuroma was not significantly different from the control, which indicates that the process of nerve trunk regeneration has actually ended.

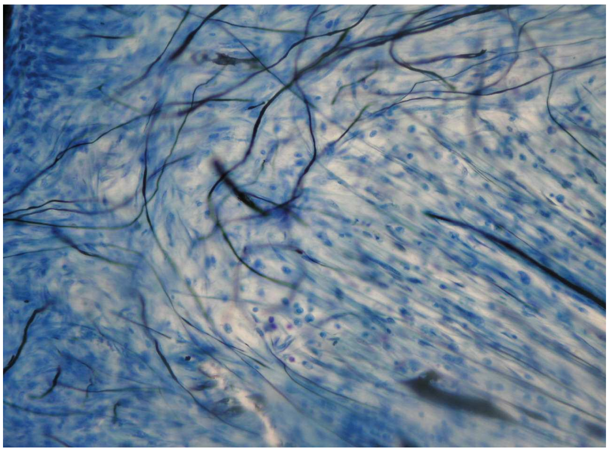

Fig. 1. Regenerating axons, newly formed capillaries and connective tissue cells in the regenerative neuroma of the sciatic nerve of the rat three weeks after the surgery. Blood vessels injection with Indian ink-gelatine, silver nitrate impregnation, azur II-eosine. x200

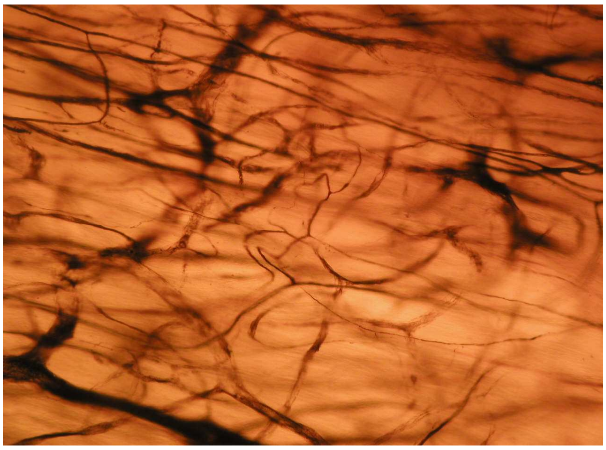

Fig. 2. Newly formed blood vessels in the regenerative neuroma of the sciatic nerve of the rat three weeks after the surgery. Blood vessels injection with Indian ink-gelatine. x100

In the early post-traumatic period the capillary density at the site of regenerative neuroma also increases. What is the reason for the growth of this indicator? In the intact nerve the heomicrocirculatory bed provides for the metabolic needs of its cellular elements (Shwann cells and epi-, peri and endoneurial cells). After neurotomy without neurography, a small diastasis (up to 1 mm) is formed between the central and peripheral stumps, which is initially filled with blood clot. Subsequently, in its place, aseptic inflammation develops and connective tissue is formed, into which axons from the central stump and the bands of Bungner from the peripheral one grow [5, 13]. The number of Schwann cells in neuroma does not exceed their number, located over 1 mm of the intact nerve, since each band of Bungner is a continuation of a column of Schwann cells of the preexisting nerve fiber of the peripheral stump. The number of the connective tissue cells grows at the expense of blood monocytes migration and proliferation of fibroblasts. However it is difficult to explain the two-fold increase in capillary density at the height of the regeneration process (six weeks after the injury) by the sole necessity of meeting the needs of macrophages and fibroblasts. That dynamics of neuroma capillary density corresponds with that of the nerve fibers is noteworthy.

In the intact nerve the processes of synthesis take place in the neuron body, and then the synthesized substances are transported to the periphery with the help of axoplasmatic transport. After injury, synthetic processes are activated, but for many years, researchers have wondered whether this activation is sufficient for ensuring the de novo formation of the nerve fibers. Zheng et al. were the first to give answer to this question [15]. In a study performed on a cell culture of the spinal ganglion, it was shown that translation processes take place in axons separated from the neuron bodies. In particular, β-tubulines necessary for the formation of microtubules in the axons themselves and their growth cones are synthesized. Blocking of translation in axons led to retraction of the growth cones. Later it was found that ribosomes and mRNA were transported to the distal parts of regenerating axons from Swann cells. Thus, the Shwann cells not only supply axons with "translational machinery", but can also modify the axon "translational products" [6]. In [12] it was demonstrated that in tissue culture Swann cells release exosomes, administration of which to the study animals had stimulated the sciatic nerve regeneration. Lopez-Leal and Court [11] confirmed these data and proved that it is with the help of exosomes that mRNA and ribosomes are transported to the regenerating axons *in vivo*. And finally in 2018 Chung [4] demonstrated that calcitonin gene-related peptide synthesized in neurons, to which the number of receptors on Shwann cells dramatically increase after the injury, is the signal to Shwann cells to release exosomes.

Conclusion

Hypervascularization of the peripheral nerve regenerative neuroma after its injury provides for the metabolic and plastic needs of the regenerating axons and connective tissue cells. This phenomenon may be used to develop methods for stimulating the hemomicrocirculatory bed and microhemorheology in the damaged nerves with the purpose of improving the outcomes of their regeneration.

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Реферати

ГІПЕРВАСКУЛЯРИЗАЦІЯ РЕГЕНЕРАЦІЙНОЇ НЕВРОМИ

Чайковський Ю.Б.

Морфологічні зміни після травми і в процесі відновлення нервових стовбурів добре вивчені. Однак кількісні дані про нервові волокна і микросудини регенераційної невроми нечисленні. Метою даного дослідження було морфометричне вивчення нервових волокон і капілярів регенераційної невроми сідничого нерва щурів через 3, 6, 12 і 24 тижні після нейротомії. Показано, що в ранні терміни експерименту відбувається значне збільшення щільності капілярів і нервових волокон в регенераційній невромі. До 24 тижнів після операції ці показники нормалізуються. Зроблено висновок про те, що гіперваскуляризація

ГИПЕРВАСКУЛЯРИЗАЦИЯ РЕГЕНЕРАЦИОННОЙ НЕВРОМЫ

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Морфологические изменения после травмы и в процессе восстановления нервных стволов хорошо изучены. Однако количественные данные о нервных волокнах и микрососудах регенерационной невромы немногочисленны. Целью данного исследования явилось морфометрическое изучение нервных волокон и капилляров регенерационной невромы седалищного нерва крыс через 3, 6, 12 и 24 недели после нейротомии. Показано, что в ранние сроки эксперимента происходит значительное увеличение плотности капилляров и нервных волокон в регенерационной невроме. К 24 неделям после операции эти показатели нормализуются. Сделан вывод о том, что гиперваскуляризация регенерационной

регенераційної невроми периферійного нерва після його травми забезпечує метаболічні і пластичні потреби регенеруючих аксонів і клітин сполучної тканини.

Ключові слова: регенераційна неврома, сідничний нерв, гіперваскуляризація, щури.

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невромы периферического нерва после его травмы обеспечивает метаболические и пластические потребности регенерирующих аксонов и клеток соединительной ткани.

Ключевые слова: регенерационная неврома, седалищный нерв, гиперваскуляризация, крысы.

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MACRO- AND MICROSTRUCTURE OF HUMAN FETUS CEREBELLUM AT THE 13-14TH WEEKS OF INTRAUTERINE DEVELOPMENT

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The study was performed to establish the structure of the vermis and cerebellum hemispheres macrometric parameters, as well as morphometric parameters and cytoarchitectonics of the human cerebellum on the 13th-14th weeks of fetal development. The anatomic-histological, immunohistochemical and morphometric study was carried out on the vermis and hemispheres of the cerebellum in 12 human fetuses with a gestation term of 13-14 weeks without abnormalities of the central nervous system, which were obtained as a result of a late abortion in the regional pathologist bureau of Vinnytsia. The study found: the transverse size of the cerebellum, longitudinal dimensions, height and transverse size of the cerebellum right and left hemisphere; transverse, longitudinal size and height of the vermis, as well as mass of the cerebellum. It is established that in the histo-cytoarchitectonics of the cerebellum hemispheres, there are three layers: the ventricular zone, the intermediate zone, the cortical zone, which in turn is divided into internal granular, molecular and external granular layers. In the course of the research it was established: the ventricular zone of the right cerebellum has the smallest thickness, and the intermediate zone has the largest thickness; the intermediate zone of the left cerebellum has the greatest thickness, and the ventricular zone has the smallest thickness; the smallest density of neural stem cells is observed in the molecular zone, and the largest is in the ventricular zone; more intensive proliferation of neural stem cells present in the ventricular zone, and less intense - in the intermediate zone; starting from the ventricular zone, the radial glial cells fibers pass through all layers of the cerebellum to the external granular layer in the radial direction; the expression of Synaptophysin is present in all layers of the cerebellum. Thus, the macrometric parameters of the cerebellum formations of 13-14 weeks fetuses are established.

Keywords: cerebellum vermis, cerebellum hemispheres, morphometric parameters, radial glia, intrauterine development.

The study is a fragment of the research project "Establishment of morphological changes in the formations of the central nervous system of a person during the prenatal period of ontogenesis (macroscopic, histologic, morphometric, immunohistochemical research)", state registration No. 0118U001043.

In the Vinnytsia region, the total number of stillbirths and late abortions over the past five years was 172, of which 99 males and 73 females. The highest frequency of pathologies of late abortions and stillbirths was observed at the age of 19-20 weeks of fetal development, which amounted to 35 cases, accounting for 20.3% of all cases in a year. The smallest number of cases was observed in the following gestational periods: 15-16, 28-29, 33-34, 41-42 weeks, which in total was 0.6%. Among the causes of late abortions and stillbirths in the Vinnytsia region over the past five years, the predominant proportion had fetal asphyxiation (ante or intranatal) - 70%. Congenital malformation was 30%. Of these, the defects of the central nervous system (CNS) (hydrocephalus, anencephaly, spina bifida, etc.) accounted for the highest percentage - 9.3% [1]. Thus, research on the development of CNS structures in ontogenesis remains a very topical issue.

In the formation of CNS structures, the leading place belongs to the processes of proliferation, migration, growth and differentiation of cells. However, scientific work that reflects morphometric parameters of the brain in the embryonic development period more often refers to the determination of sonographic or MRI parameters of the fetus. Therefore, for a more detailed study of mechanisms of embryogenesis and understanding mechanisms of the pathogenesis of birth defects, there is a need for certain morphometric (histometric) parameters of the brain organs in different gestational periods.

The development of skull bones is interconnected with an increase in the growth rate of the brain, so congenital malformations of the CNS structures are often combined with craniosynostoses, and also, in craniosynostoses, there are liquor-dynamic disorders that cause changes in the structure of the brain developing during the period of fetal development [8].

Taking into account the development and improvement of medical technologies and diagnostic methods, the interest of neuromorphologists, neurosurgeons and neuropathologists, as well as specialists