THE RESULTS OF THE STUDY OF THE PARANEURIUM CELLULAR COMPOSITION OF PERIPHERAL NERVES FROM THE BRACHIAL PLEXUS IN THE MIDDLE THIRD OF THE SHOULDER IN THE PHYLOGENETIC RANGE OF ANIMALS

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We conducted our study on 120 neurovascular fascicles of peripheral nerves from the brachial plexus in the middle third of the shoulder on the medial surface of the limb. Received tissue specimens were stained with hematoxylin-eosin according to the method of Van Gison and Mallory. The research of epi- and paraneural connective tissue of neurovascular fascicles was held among representatives of the following classes: Amphibians, Reptiles, Birds and Mammals. As a result, it was revealed the prevalence of a number of fibroblast cells over other cells of the connective tissue. The quantity of non-resident cells (lymphocytes, mast cells) and their density on the standard square of section is enlarged in process of complicating the function, which is carried out by an extremity. The degree of maturity of the connective-tissue fibers, their density and quantity also increases. Existence of strong positive correlation between the area of paraneurium and the area of all neurovascular fascicle proves the direct dependence of these indicators.

Keywords*: epineurium, paraneurium, connective tissue stroma of the peripheral nerves, neurovascular fascicle, resident cells, non-resident cells*

Introduction

Despite the reduction of total injury indicators over the last five years, the number of injuries involving violation of the nerve trunks integrity remains at a high level [4, 6, 7]. Thus upper limb injuries make up 1/3 in structure of all damages of the musculoskeletal system [1, 2, 5]. Full function of the upper limb has a particular importance for man as an organ of work and fine-coordinated activity. Malfunctions of the upper extremity as a result of injuries lead to long-term disablement and disability in the active age. For the correct and appropriate treatment of such lesions we require more complete and actual knowledge about the features of the microscopic structure of not only stromal, but also conductive component of the peripheral nerves [3, 8]. In this regard we have an increasing need for an information base about the morphofunctional state of connective tissue, which surrounds peripheral nerves.

The aim of the study is to investigate the morphological features of epi- and paraneural connective tissue of peripheral nerves from the brachial plexus in the middle third of the shoulder on its medial surface in the phylogenetic range of animals.

Materials and methods

The study was conducted on the peripheral nerves from the brachial plexus in the middle third of the medial surface of the shoulder. This material was received from the forelimbs of amphibians, reptiles, birds and mammals. For the manufacture of tissue specimens, we dissected the neurovascular bundles with surrounding muscles in the middle third of the shoulder on its medial surface. The received material, after washing and dewatering, was embedded in paraffin by a reference technique. Then we made microscopic sections (10-12 microns thick) with the help of sledge microtome and stained them with hematoxylin-eosin according to the method of Van Gison and Mallory. Further received tissue specimens were photographed by using an optical system, which consists of a microscope Leica CME and the eyepiece-camera DCM-510, with magnifications x40, x100, x200 and x400. All images were documented in the program FUTURE WINJOE, supplied eyepiece-camera. The shape and topography of neurovascular bundle and its components as well as the presence and severity of the paraneural structures was evaluated at low magnification microscopy. The color intensity of the fibrous component and fibers ordering in epi- and paraneurium define the degree of manifestation of connective tissue. These characteristics are clearly visible on pictures taken at high magnification. Moreover, the cross-sectional area of the neurovascular bundle and the cross-sectional area of the surrounding connective tissue were measured on the micrographs. The study of epi- and paraneural connective tissue cellular composition was carried out by finding karyological features on the standard cut square. The number of resident cells (fibroblasts, fibrocytes, macrophages) and non-resident cells (plasma cells, mast cells, white blood cells - monocytes, lymphocytes, PMN), expressed in absolute units, was counted on the standard cut square in one hundred fields of view. After that, for objectification the data about the condition of epi- and paraneural cellular component, we calculated cell index by the following formula:

$CI = \frac{\sum \mathrm{Fb;\ Fc;\ Mph}}{\sum \mathrm{PC;\ MC;\ Mon;\ Lph;\ N}}$, where CI is cell index, Fb - fibroblasts, Fc - fibrocytes, Mph – macrophages, PC - plasma cells, MC - mast cells, Mon - monocytes, Lph - lymphocytes, N - polymorphonuclear neutrophils [8].

The obtained data was processed with statistical methods. We determined minimum and maximum values, the arithmetic mean (M) and its error (m) and standard deviation (σ) for the studied parameters. The Spearman correlation coefficient (r) was also calculated. The significance of average differences was assessed by the criterion of the non-parametric statistics Mann-Whitney (U). All calculations were performed by using the analytical package of application Excel Office 2010. KSMU of Health Ministry of the Russian Federation has a license to use this program.

Results and discussion

As a result of the conducted research the following data were obtained: all the test animals have a triangular shape of the neurovascular fascicle in cross section. These fascicles are surrounded on two sides by muscles, which provide bending limbs; on the third side they are covered by skin with subcutaneous tissue. The severity of hypodermis depends on the stage of the animal evolutionary development. The structure complication of the connective tissue apparatus of peripheral nerve is in direct dependency with the complexity of the forelimb function. Amphibians and reptiles have not expressed paraneural structures. During the microscopic study of surrounding birds' nerve trunks connective tissue, we revealed that the pigeons have well-defined connective paraneural case. This structure provides the functions of depreciation during the flight, due to the presence of its own blood vessels - an additional power supply of the nerve trunk. Such adaptive changes explained considerable physical strain on the limb during the flight, in comparison with the flightless birds.

After morphometric research it was found out that the area of cross section of the neurovascular bundle had a reliable tendency to increase ($p \leq 0,05$) as much as area of cross section of surrounded connective tissue. For instance, such area for Amphibians is $0,362257 \pm 0,021243$ mm^2 for the left limb and $0,38122 \pm 0,019052$ mm^2 for the right limb, for Mammals it was more in 10 times. It was observed that the quantity of cells increased for each unit of the area of cross section, what regarded the density of fibers in connective tissue too, it increased as well. Compared animals from one class, we revealed that the measure of coloring and order of fibres in connective tissue was the biggest for doves and foxes.

We analyzed all types of cells in cross sections of medial brachial surface in its middle third for observing animals. It had been revealed that there was variety of cells types together with constant cells of fibroblasts (table 1). For example, there was almost equal quantity of mechanocyties, fibrocytes, histiocytes among Amphibians and Reptiles. A similar pattern in the quantitative content of the resident cells observed among the representatives of the Birds' class.

The representatives of the Mammalia class, Predatory order have a number of fibroblast cells more than half of the total. Analyzing the quantity of non-resident cells, we observed that there was no reliable difference in the amount of mast cells between various animal classes. The number of lymphocytes was twice more for Birds and Mammals. By the way, the reliable difference ($p \leq 0,05$) was for a dove and a fox especially. The number of plasma cells, neutrophils and monocytes didn't have any reliable differences; it varied from 2% till 5% in different species of animals.

Table 1

The cell variety of epineural and paraneural connective tissue of peripheral nerves from brachial plexus in the middle third of the shoulder applied on phylogenetic range of animals (%)

CELL NAME	AMPHIBIANS (FROG)				REPTILES (LIZARD)				BIRDS								MAMMALS							
									DOVE				HEN				FOX				DOG			
	LF	RF	LE	RE	LF	RF	LE	RE	LF	RF	LE	RE	LF	RF	LE	RE	LF	RF	LE	RE	LF	RF	LE	RE
FIBROBLASTS	27	26	29	27	27	25	29	22	22	24	22	21	22	21	19	20	35	33	32	34	30	30	27	29
FIBROCYTES	16	22	17	22	16	18	15	20	20	21	21	23	20	19	20	20	21	20	19	18	20	22	26	25
MACROPHAGES	20	21	22	22	21	20	21	23	20	20	23	22	21	19	21	22	11	13	13	14	13	12	11	11
MAST CELLS	20	18	20	19	20	22	21	25	18	17	17	16	16	18	19	19	22	20	20	19	21	21	19	20
LYMPHOCYTES	7	9	6	7	7	7	6	5	11	12	7	8	12	14	14	13	11	10	12	13	12	13	11	12
PLASMA CELLS	3	2	1	1	2	3	1	0	4	2	4	5	3	4	4	5	0	2	2	0	1	0	2	0
MONOCYTES	3	0	2	1	2	2	2	0	2	2	3	3	4	3	0	1	0	1	0	1	2	0	0	1
PMN	4	2	3	2	5	3	2	1	3	2	3	2	2	2	3	0	0	1	2	1	1	2	3	2

*LF is left flexor; RF – right flexor, LE - left extensor, RE - right extensor

We counted cell index and came to conclusion that its number was more for animals with active moving style such as a dove and a fox; it was a true evidence of collagenesis progress and growth of surrounded connective tissue. We carried out correlation analysis between the area of cross section of neurovascular bundle and square of surrounded epineural and paraneural connective tissue; we counted Spearman's correlation coefficient. Its number was 0,81-0,98 for all observing animals, which meant that correlation was strong. The relationships between parameters were positive; the square of surrounded connective tissue was increasing as much as area of cross section of neurovascular fascicle, and it concerned also the degree of its development.

Conclusion

During the morphometric research we revealed constant and continuous differences in the structure of stromal apparatus of peripheral nerves from the brachial plexus in phylogenetic range of animals by the influence of complication of extremity organization. The differences were: complications of stromal structure of peripheral nerves from the brachial plexus, increasing the absolute number of fibroblasts and fibrocytes in paraneural stratum; growing number of non-resident cells; positive relationships between the area of connective tissue and the square of neurovascular bundle. In summary, the P. Lesgaft's statement of unity of form and function had been proved again.

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