CLINICAL-MORPHOLOGICAL PECULIARITIES OF THE PERIPHERAL NERVOUS SYSTEM IN DIFFERENT TYPES OF THE MULTIPLE SCLEROSIS COURSE

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Резюме: при исследовании в элементах периферической нервной системы пациентов с рассеянным склерозом выявлено демиелинизация и дистрофические изменения нервных волокон в виде разволокнения, склероза, очагового уменьшения шванновских клеток, а также воспалительные изменения с экспрессией большого количества циклооксигеназы-2 и индуцибельной фракции синтазы оксида азота.

Summary: in the study of peripheral nervous system structures in patients with multiple sclerosis, demyelination and dystrophic changes of nerve fibers were revealed in the form of defibration, sclerosis, focal shrinkage of Schwann cells, as well as inflammatory changes with the expression of large amounts of cyclooxygenase-2 and inducible fraction of synthase nitric oxide.

Relevance. Multiple sclerosis (MS) is a progressive autoimmune disease of the central nervous system characterized by local infiltration of T cells and macrophages into the nerve tissue, local multiple sites of inflammation, glial activation, damage of the oligodendrocytes, intensive demyelination of nerve fibers, axonal damage and severe neurologic impairments [3].

The majority of unsolved problems in this pathology is due to the lack of both clear-cut ideas about the etiology and pathogenesis of MS and reliable criteria for its diagnosis. Previously it was assumed that the centers of demyelination in MS do not spread from the central nervous system (CNS) to the periphery through the transition zones, where glial cells give way to Schwann cells and fibroblasts. Recently, more and more data have appeared that the lesion of the peripheral nervous system (PNS), in the form of distal hypotrophy, and sensitivity disorders of mono- and polyneuric type, are often found in MS [2]. However, very often decline of the function of the PNS develops on the background and in the interrelationship with the lesion of the central nervous system in the clinical picture.

It has been established that a very wide spectrum of cytokines and proinflammatory factors is synthesized in MS, among them interleukins 1-4, 6, 10, 12, TNF-α, interferons, etc. In recent times, the role of inflammation in damage of nerve structures in MS has been proven [3]. One of the leading markers of inflammation is cyclooxygenase-2 (COX-2), an inducible enzyme that stimulates the production of pro-inflammatory prostaglandins [1], its role in the complex cascade of the pathogenesis of MS remains poorly studied [5, 7]. There is an increasing evidence of the possible role of endothelial dysfunction and impaired metabolism of nitric oxide (NO) in MS [6]. NO, is a key regulator of vascular, nervous and immune homeostasis. However, at high concentrations, this mediator can exert a cytostatic effect.

The presence of information of the damage of the PNS in MS and the lack of understanding of the pathological processes occurring in its structures, leading to irreversible changes, led to the relevance of this study.

The aim of the study was to investigate the expression of COX-2 and inducible isoform of NO synthase in PNS structures and skin vessels in patients with MS on the basis of biopsy.

Materials and methods. The material for the histological and immunohistochemical study was biopsy of the skin of 32 patients with veryfied diagnosis of multiple sclerosis according to the McDonald criteria [8]. In 5 patients, primary-progredient type of MS course was observed, 7-secondary-progredient, 13-recurrent-remitting in remission, and 7-relapsing-remitting in the stage of exacerbation. As a control, skin biopsies of 7 patients with a surgical profile without neurological diseases were studied.

Skin biopsies were taken in the place of the lower third of the inner surface of the ankle under local anesthesia with 0.5% solution of novocaine. The entire material was fixed in a 10% solution of neutral formalin. Then biopsies were subjected to standard wiring through the alcohols of increasing concentration, Nikiforov's liquid (96% alcohol and diethyl ether in a 1: 1 ratio), chloroform, and then paraffin. Of the blocks prepared in this way, serial sections were made with a thickness of 4-5 × 10-6 m. Paraffin sections were stained with hematoxylin and eosin, by Spilmeyer and impregnated with silver nitrate by Bilshovsky-Gross. Expression of iNOS and COX-2 by indirect immunohistochemical peroxidase method using monoclonal antibodies (MCAB), followed by incubation with diaminobenzidine and staining with Mayer's hematoxylin was studied. Histological and histochemical methods were performed according to the prescriptions given in the manuals on histological technique and histochemistry.

The intensity of expression of NO syntheses and COX-2 was possessed by cytophotometry, determining the optical density of immunopositive granules in the green portion of the spectrum. In each observation 3-5 randomly chosen fields of vision were studied.

The complex of histological and cytotoxicometric studies on an Olympus BX-41 microscope using Olympus DP-Soft (Version 3: 1) and Microsoft Excel were performed .

Results of cytophometric and immunohistochemical data were processed by mathematical statistics using variational analysis. Using the methods of alternative and variational statistics, we calculated the arithmetic mean, the standard deviation, the average error of the difference, the probability of the difference. The probability of a difference between the two averages was determined from the Student's table [4]. In determining the degree of probability, an accuracy of $p \leq 0,05$ was assumed, which corresponds to $P \geq 95.0\%$.

Results of the study. A survey microscopic study of biopsy specimens of patients with MS stained with hematoxylin and eosin revealed signs of productive vasculitis, which was manifested by widespread perivascular and perineural lymphocytic infiltration. Vessels of all calibers with swollen endothelium, perivascular spaces sharply widened, in some cases hypertrophy of the muscular layer, corrugation of the elastic membrane and its invaginates into the lumen of the vessels with focal proliferation of the endothelium were

revealed. Collagen fibers of the dermis, in immediate proximity to edematic perivascular space, swollen, homogenized and poorly stained with eosin, and by van Gison - picrinophilic were detected.

With a detailed study of the cellular nature of the inflammatory infiltrate, we found that the bulk of it was composed of lymphocytes and monocytes. When carrying out the immuno-peroxidase reaction with MCAB to COX-2 and iNOS in the cells of the inflammatory infiltrate, endothelium of the vessels and the muscular layer, the expression of these enzymes was revealed. It should be noted that the most pronounced morphological manifestations of vasculitis in the group of patients with relapsing-remitting type of MS course in the acute stage, as well as in the group with primary-progredient and secondary-progredient types of MS flow with a duration of up to 5 years were observed.

Also noteworthy is the fact that the earliest and massive infiltrates appeared around the vessels in the area of the neurovascular bundles. Subsequently, the number of cells around the vessels decreases, and around the base of the single-going nerves increases. However, even with the disappearance of the phenomena of vasculitis, in the endothelium, muscle layer and perivascular space, the expression of iNOS granules was determined. While studying the components of the PNS, lymphocyte infiltration around the nerve wires were seen. Some nerve fibers were sharply edematous, focally unstructured, swollen with discharged of the base and presence of fields of complete absence of Schwann cells. Perineural spaces are greatly expanded. Directly in the nerves lymphocytes, expressing COX-2 and iNOS were determined .

When the tissue was impregnated with silver nitrate, thickening and coarsening of the nerve fibers, uneven coloring with the phenomena of varicose-like swelling of the neurolemmus, and the formation of clavate blisters (kugel-phenomena) of nerve endings were noted.

When painting using the Shpilmeyer method, the myelin sheaths and axial cylinders were stained, with focal fragmentation, the presence of aneurysm-like extensions, and the intermittent staining of the axial cylinders, indicating neurodegenerative processes.

During the immunoperoxidase reaction with MCAB to COX-2 and iNOS, the activation of these enzymes in Schwann cells of nerve fibers was detected. The granules of enzymes were distributed in the cytoplasm of cells. It should be noted that the quantity of immunopositive granules iNOS many times exceeded the quantity of such COX-2. The enzymes investigated near the nerves were detected in the vessel wall, as well as in the interstitial space of the dermis, far from the vascular bed and nerves. The iNOS granules throughout the nerve fiber were distributed, while the COX-2 pellets had a mosaic occurance and concentrated more on the outer surface of the nerve fiber.

The most pronounced and acute morphological changes in the peripheral nervous system in the group with a relapsing-remittent type of course within the exacerbation stage were noted, and also in the group with the primary-progredient and secondary-progredient type of RS course with the duration disease up to 5 years. In patients with progredient course and with experience of MS for more than 5 years the morphological pattern of chronic dystrophic and sclerotic changes was revealed. At the same time, the expression of COX-2 and iNOS was decreased, which, apparently, related to the death of neurolocytes,

synthesized these enzymes.

It should be noted that sclerotic and dystrophic changes directly correlate with the severity of persistent neurological deficit.

In the group with a relapsing remitting course in the stage of remission, morphological changes were minimal. There were no expressed atrophic and sclerotic changes in this study group.

A cytophotometric study of the optical density of COX-2 the significant increasing of this indicator for MS as a whole have revealed. However, it should be noted that COX-2, as well as iNOS, is an inducible enzyme, that is why, it appears in pathological conditions, like, inflammation. Optical density in this situation was considered as a quantitative indicator reflecting the activity of the inflammatory process. In the control group, the expression of this enzyme was not determined.

When studying the optical density of iNOS, depending on the type of MS course, it was noteworthy that the highest optical density values were determined in the group with the remitting-relapsing type of MS course in the exacerbation stage and were 0.217 ± 0.008 conventional units. Less mean values in groups with primary-progredient and secondary-progressive types of MS course were 0.171 ± 0.009 conventional units and 0.157 ± 0.011 conventional units accordingly. The lowest average value of the optical density of iNOS granules in the group of remitting-recurrent type of MS course in the remission stage amounted 0.056 ± 0.017 conventional units was detected.

Thus, the highest values of COX-2 optical density in the group with remitting-recurrent type of MS on the stage of exacerbation were observed, amounted to 0.136 ± 0.007 conventional units. Practically identical indices of optical density in primary and secondary-progressive types of MS were observed, amounted to 0.118 ± 0.008 conventional units and 0.115 ± 0.01 conventional units accordingly. The lowest values of COX-2 optical density in the group with the remitting recurrent type of MS in the remission stage ammounted 0.051 ± 0.014 conventional units were observed.

Analyzing the optical density of COX-2 and iNOS in the group with the remitting recurrent type of MS course in the remission stage, it should be noted that, despite the presence of clinical remission, the inflammatory and dystrophic processes continue to occur in PNS components. When analyzing the homogeneity of the optical density indices of COX-2 and iNOS in the group with the remitting-recurrent type of the MS course in the stage of remission, a rather pronounced dispersion of these indices were noted. Thus, fluctuations in the optical density within the group varied from 0.01 conventional units up to 0.076 conventional units, which may reflect the depth of clinical remission, and, possibly, a predisposition to exacerbation and further progression of the disease.

Conclusions.

1. In the structures of PNS at MS, demyelination and dystrophic changes of nerve fibers occur in the form of defibration, sclerosis, varicose veins and focal shrinkage of Schwann cells.

2. In the perineural and perivascular spaces of the skin, inflammatory changes were detected. In the cells of the lymphocytic inflammatory infiltrate the big amounts of COX-2 and iNOS were xpressed.

3. One of the link of pathogenesis, which enhances demyelination and dystrophic

changes in nerve fibers, can be activation of iNOS and COX-2 in Schwann cells .

4. The presence of endothelial dysfunction and inflammatory changes in PNS components of different types of MS course, including in the remission phase, should be considered prescribing pathogenetic therapy.

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