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INFLUENCE OF SENSITIZATION WITH BRAIN ANTIGEN SENSITIZATION ON THE CONDITION OF CEREBRAL CORTEX SENSOMOTOR NEUROGLIAL ELEMENTS OF THEIR IMMUNOHISTOCHEMICAL DETECTION

Abstract. *Conducted studies in rats revealed, that sensitization with the brain antigen is the factor, which can lead to the brain damage and induce the neuroinflammation, gliosis and neurodegenerative processes on its own. Evaluation of expression of GFAP, S100 and Iba-1 in the cerebral cortex makes it possible not only to determine the change in number of glial cells, but also, to some extent, to estimate their functional activity.*
Key words*: brain, sensitization, GFAP, S100, Iba-1*

Introduction. Normally, cerebral tissue is isolated from immune system with blood-brain barrier [15]. But, due to the data of several authors, from 5 to 92% of people have antibrain antibodies in their blood [5,9]. Their presence can lead to the brain damage, initiate or intensify neurological processes [3, 4, 5, 9, 13], which can be accompanied by toxicity and glial neuroinflammation [12]. Under these conditions both degenerative changes of neurons and multidirectional reactive changes of glia may appear [8, 13, 14].

Objective: to find out influence of sensitization with brain antigen on the state of glial cells in sensomotor cerebral cortex at their immunohistochemical detection (S-100, GFAP, iba-1).

Materials and methods. Study was performed with 35 white pubertal male Wistar rats with the body weight of 260-290 g. The animals were held in vivarium, fed a standard diet and housed 5 animals in a cage with free access to food and water and under constant light conditions due to the «Principles of Laboratory Animal Care». Study was performed in strict accordance with «Guide for the Care and Use of Laboratory Animals» (NIH publ. No. 93 23, revised 1985). The male rats were used in the study as the estrogen level influences the course of the brain damage considerably [11]. The experimental animals were sensitized with 20% water-salt extract of the homologous brain tissue, prepared by the standard procedure [2], and containing of 0,33-0,5 mg/ml protein by Lowri. The extract was subcutaneous intro-duced into the rats as follows: 1st day - in amount of 0.5 ml; 2nd day - 1 ml; 3d day - 1.5 ml [1]. The control group is 10 animals, which were not under any action.

The brain was studied in 12, 15, 22, 42 and 102 days after the start of the experiment with the introduction of the sodium thiopental (200 mg/kg) in animals. During up to 1 minute the skull was opened and the brain was removed and sectioned frontally into three pieces, then the middle piece was placed in the 10% buffered formalin (pH 7.4, 40oC) for 24 hours. The samples were placed into the paraffin, and the slices with thickness of 4 µm were produced with the following staining with azure II-eosin and phosphotungstic hematoxylin.

Immunohistochemical (IHC) reactions were performed according to the manufactures` protocols with the antibodies against: protein S-100 (Polyclonal Rabbit Anti-S100 (Dako, Denmark)), Iba-1 (rabbit polyclonal, 1:750, Molecular Probes Inc., USA), GFAP (Dako, Denmark). In order to visualize the products of the IHC-reaction the system EnVisionTM FLEX, (Dako, Denmark) was used. The slices were stained with hematoxylin Gill. The samples of the

rats brain with defined reactivity were used as a positive control. As a negative control were used the samples, obtained without using the primary antibodies.

Tissue specimens were studied and photographed with the microscope Ni-kon Eclipse 80 and camera DS-5SMc/L2 (Nikon, Japan) under standardized con-ditions. GFAP+ and Iba-1+ cells were counted at 10 areas 430x320 µm. For densitometry evaluation of the expression of S100 in digital images (x400, 1280x960 pixels RGB) the system of analysis ImageJ 1.46 was used. Obtained digital data were processed by standard statistical methods.

Results and discussion. Conducted studies showed, that in 12, 15 and 22 days after the sensitization the mild perivascular edema takes place in the cerebral cortex. Neuron contours are usually irregular. Often the phenomena, which can be described as hypercondensation of chromatophilous substance is observed. Furthermore, there are some cells of chromatolysis in the cytoplasm of neurons. Degenerative hyperchromic neurons occur rather often, necrotic neurons occur less frequently, and sometimes the shrunken neurons with clarified cytoplasm occur. In some cases there are cells of small spongy degeneration. Later on, these effects become less expressive, but the increase in the number of the glial cells appears. In 102 days after immunization small clusters of glial cells sometimes appears in the cerebral cortex.

Evaluation of the expression of GFAP in the sensomotor cerebral cortex of rats of the control group revealed the cytoplasmically marked cells with processes. They have little nuclei, surrounded with a thin layer of cytoplasm, and thin processes, which moderately branch and gradually thin. Such cells structure, revealed by the expression of GFAP, most closely matches the macroglia of CNS [6, 7]. At the same time numerous small granular and filamentous GFAP + formations are also detected in neuropile. These formations can be considered as the fragments of astrocytes, which are in the slice.

For the sensitized animals there is some increase in the number of the GFAP+ cells in 12-15 days after the start of the experiment. At the same time their bodies and processes often seem edematous. Usually there is a higher level of expression of GFAP in these samples compared to the control ones. In addition, the thinnest terminal branches of processes were visualized something worse than in control samples. Under these conditions there is smaller number of the GFAP+ cells in the neuropile. Later on, with a general increase in the number of glial cells in the sensomotor cerebral cortex, there is an increase in the number of the GFAP+ cells, that is approved by evaluation of their specific number (Figure). Usually the cells are something hypertrophied.

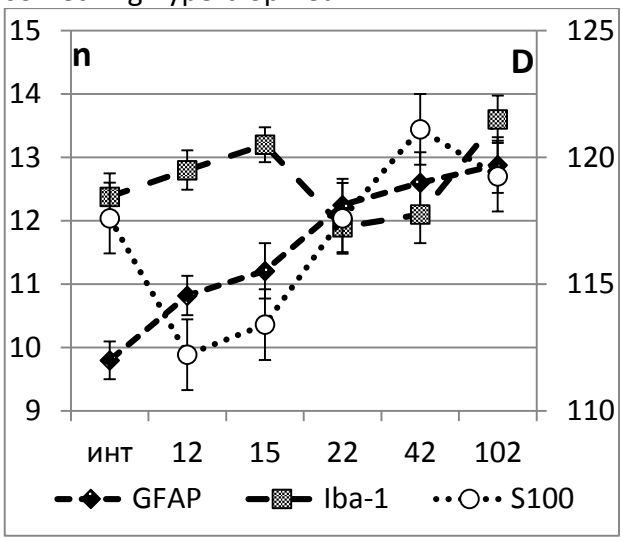

Figure. Change of number of GFAP+ (astrocyte), Iba-1+ (microglia) of cells (n – average number at the area 430x320 µm) and intensity of the expression of S100 (D, arb. units) in the sensomotor cerebral cortex of rats for different time after sensitization with the brain antigen.

For the rats of the control group the expression of the S100 appears as a small granulation, which is almost equally distributed in the neuropile. Neurons of the cerebral cortex don`t express the protein and have sharp contours, some-times they have clearly visible axon hills and apical axons. Glial cells are visual-ized just sometimes, and their bodies and processes can be seen, while their color intensity is almost as the color intensity of the surrounding neuropile. This was the reason for densitometric evaluation of the optical density of the expression of S100, instead of counting of cells.

Generally, there is a decrease in the expression of S100 in the cerebral cor-tex under

the sensitization in 12-15 days comparatively to the control samples (Figure). Marked glial cells with a small amount of the slightly branched processes are clearly seen. On the 22nd day of the experiment level of the expression of S100 is equal to the initial level (Figure). But in this case in 102 days after the start of the experiment there are marked glial cells, that indicates the higher ac-cumulation of S100 in their bodies and large processes than in terminal branches of the processes.

Revealed Iba-1+ cells of rats of the control group have rather small nuclei and processes and typical structure of microglial cells. They had small amount of the slightly branched processes with thin branches. Sometimes these cells are ad-jacent to blood microvessels or neurons along the perimeter.

Under the sensitization number of the Iba-1+ cells in 12 and 15 days after start of the experiment is something higher than in control samples (Fig. 1). But intensity of their marking is something lower and appears less intensively than for their processes. On 22nd and 43d days of the experiment number of the microglial cells decreases and does not differ from control values significantly. At the same time there is some increase in the intensity of the expression of the Iba-1. In 102 days after the start of the sensitization in the sensomotor cerebral cortex there is much higher number of the microglial cells with the much higher level of the expression of Iba-1 comparatively to the control samples. Also the cells usually have bigger dimensions and higher number of the processes than in the con-trol samples.

Thus, the studies that were carried out, can be considered as analogues of the sensitization with brain antigens under some pathological states [4, 5, 9, 13]. It was shown, that mild neurodegenerative processes take place and expressive reaction of glia develops even at the absence of the direct vascular brain damage. The reaction has the character of neuroinflammation with the increase in amount of Iba-1+-microglia and its hypertrophy. At the same time there is a gliosis with the gradual and dynamically stable increase in the number of astrocytes and their hyperplasia, which is clearly revealed by the expression of GFAP. Mostly this process coincides with the increase in the antibrain antibodies number in the blood of the experimental rats [3]. The decrease in the expression of S100, which allows estimating of the degree of differentiation and functional activity of the glial cells [10], and also, to some extent, of GFAP in the cells of microglia of the sensomotor cerebral cortex at the early stage of the development of sensitization (during the first month), indicates the decrease in their activity. The increase in the number of the cells of microglia as a response to the sensitization is nonlinear, and mostly coincides with the dynamics of changes in circulating immune com-plexes in the blood of experimental animals [3].

Conclusion. Sensitization with the brain antigen is the factor, which can lead to the brain damage and induce the neuroinflammation, gliosis and neurodegenerative processes on its own. Evaluation of expression of GFAP, S100 and Iba-1 in the cerebral cortex makes it possible not only to determine the change in the number of glial cells, but also, to some extent, to estimate their functional activity.

Prospects of further research. Further studies in this area may be aimed at the understanding of the ideas on the role of the immune system in the development of morphological and functional changes of the gangliar layer of the sensomotor cerebral cortex.

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