Evaluation of Rat’s Brain Morphology Following the Induction of Acute Meningitis Treated with Ceftriaxon

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Abstract
The brain, which is the center of our coordination, has protective layers around it to protect its soft and delicate tissue, and if any of these layers are disturbed, a person will face a complicated situations and serious health problems. The brain has three protective layers of bone or skull tissue, the blood tissue layer, and finally the meningeal layer. The layer of blood tissue is the blood vessels that are located between the skull and the meningeal membranes. If germs or foreign matter enter the fluid through the blood vessels in any way and cause infection, the bones that protect the meninges will break and cause tissue damage, or any other factor that can cause inflammation of these membranes can occur. The aim of the current study was to study the histological and immunohistochemical characteristics of the brain of rats that underwent induced acute purulent pneumococcal meningitis after antibiotic therapy with Ceftriaxon. Twenty white adult male Wistar rats, were divided into three groups. The first group included 5 animals as the control group. Control group were injected with a saline solution into the subarachnoid space in an equivalent amount. The second and third groups of rats, 5 and 10 animals, respectively, were simulated acute purulent meningitis by injecting 10 μl of S. pneumoniae suspension into the subarachnoid space of the brain using a 23-G needle. Various areas of the brain of rats in acute experimental meningitis caused by S. pneumoniae after treatment with Ceftriaxon were examined. The S. pneumoniae culture was injected into the subarachnoid space in the area of the rhomboid fossa. Treatment started 18 hours after the injection. On day 10, repeated puncture was performed with analysis of cerebrospinal fluid in order to confirm the absence of meningitis, after which the animals were taken out of the experiment. No signs of meningitis were found on histological examination. Mild perivascular and pericellular focal edema with signs of overload of the lymphatic system of the brain, focal ischemic changes in neurons were revealed.
Investigation of expression with caspase 3 revealed a positive reaction of individual neurons. A positive reaction with antibodies to NeuN and Doublecortin was detected in most neurons, GFAP-positive astrocytes and their processes were visualized in all layers of the brain substance. The reaction with NSE, MAP-2, CD 31 and CD 34 is negative. Typical structure and pictures revealed an unchanged brain and purulent meningitis in the first and second groups. The microscopic image as well as the changes revealed during immunohistochemistry by dual corticosteroid antibodies and neuronal nuclear protein are characterized by predominantly cytoplasmic and perinuclear reactions, respectively. Some neurons are positive for caspase-3 and are related to changes in the characteristic of premature aging.

**Keywords:** Acute meningitis, Ceftriaxon, Immunohistochemical markers, Rats’ brain

1. Introduction

Acute meningitis is an inflammation of the leptomeninges of an infectious origin, accompanied by fever, intoxication, increased intracranial pressure, and inflammatory changes in the cerebrospinal fluid. In terms of prevalence, it ranks second after meningococcal meningitis and often occurs with the involvement of the white and gray matter of the brain in the form of meningoencephalitis (1), which leads to activation of caspases, damage to mitochondria, disruption of cellular homeostasis and initiation of apoptosis (2). Although meningitis is most often caused by a viral infection, it can also be caused by bacterial or fungal infections (3, 4). Sometimes meningitis improves within a few weeks without treatment. Of course, some meningitis cases are life-threatening and require emergency treatment with antibiotics (5-7). Bacterial meningitis is a serious problem and can cause death within a few days if not treated promptly with antibiotics. Delay in treatment increases the risk of permanent brain injury or death (8, 9).

Different types of bacteria can cause acute bacterial meningitis, the most common of which is *Streptococcus pneumonia* (Pneumococcus) (10, 11). This bacterium is the most common cause of bacterial meningitis in infants, young children, and adults (12, 13). This bacterium is more likely to cause pneumonia or ear and sinus infections (14). Vaccination can help prevent infection caused by this bacterium (15, 16). Another type is *Neisseria meningitis* (meningococcus). This bacterium usually causes an infection of the upper respiratory tract, but in cases where it enters the bloodstream, it can also cause meningococcal meningitis. It is a highly contagious infection that affects most adolescents and young adults. The infection can cause local epidemics in university dormitories, boarding schools, and military bases. Vaccination can help prevent infection caused by this bacterium (17-19).

Viral meningitis is usually mild and often resolves on its own. Most cases are caused by infection with a group of viruses called enteroviruses, and most occur in late summer and early fall. Viruses
such as herpes simplex virus (Apes), AIDS, mumps and some other viruses can also cause viral meningitis (20, 21). Underdeveloped organisms that attack the membranes and fluid around the brain also cause chronic meningitis. Chronic meningitis occurs over two weeks or more. The symptoms of chronic meningitis are similar to those of acute meningitis (22-24). Another type of meningitis is fungal meningitis. Fungal meningitis is relatively uncommon and causes chronic meningitis. Sometimes, this type of meningitis is similar to bacterial meningitis. Fungal meningitis is not contagious (25, 26). Cryptococcal meningitis is the most common form of this type of meningitis, which affects people with immune system defects, such as AIDS. This type of meningitis can be life threatening if left untreated with an antifungal drug (23, 27-29).

The nuclear protein of nerve cells NeuN is localized in the nuclei and perinuclear cytoplasm of most mammals, as well as in dendrites, but with a lower intensity of expression in immunohistochemical studies (30). The NeuN protein is often used as a marker of postmitotic neurons (31). Proteins associated with microtubules are located in the perikaryon, dendrites, and the initial section of the axon, are neuronal markers, and are studied during stem cell differentiation and transplantation; however, changes in these markers, as well as many others, have been insufficiently studied in combination with meningitis (32).

It is known that acute meningitis can be complicated by encephalitis, followed by long-term rehabilitation and recovery, and is accompanied by morphological changes in the white and gray matter of the brain. In this regard, the aim of the study was formulated to study the histological and immunohistochemical characteristics of the brain of rats that underwent experimental acute purulent pneumococcal meningitis after isolated antibiotic therapy.

2. Material and Methods
The material of the study included 20 adult male Wistar rats. The animals were divided into three groups. The first group included five animals as the control group. Control group were injected with a saline solution into the subarachnoid space in an equivalent amount. The second and third groups of rats, 5 and 10 animals, respectively, were simulated acute purulent meningitis by injecting 10 μl of S. pneumoniae suspension into the subarachnoid space of the brain using a 23-G needle. Antibacterial treatment started 18 hours after the start of the experiment with Ceftriaxon at a dose of 100 mg / kg of body weight for 7 days. The absence of meningitis was confirmed by bacteriological examination of the cerebrospinal fluid. The animals were taken out of the experiment after 24 hours and on the 11th day. Microscopic examination and photo protection were performed on preparations stained with hematoxylin and eosin after standard histological tracing using a Nikon Eclipse Ni microscope and Nis-Elements BR 4.60.00 software. For morphological examination, we took the brain, it was fixed in 10% neutral buffered formalin for 24-48 hours. After that, the preparation was
subjected to standard wiring on a Leica TP 1020 apparatus, embedded in paraffin, sections with a thickness of 4-5 μm were prepared and stained with hematoxylin and eosin, as well as toluidine blue in the Nissl modification, using standard protocols and techniques on Leica EG 1150 H apparatus, Leica RM 2245, Leica autostainer XL.

Immunohistochemical reactions were performed with antibodies GFAP (clone EP672Y, CellMarque), NSE (clone MRQ55, CellMarque), p53 (clone DO7, CellMarque), Ki 67 (clone NE14, Biogenex), NeuN (polyclonal antibodies, CellMarque), MAP-2 (clone M13, CellMarque), Doublecortine (clone 2g5, CellMarque), Caspase-3 (clone EP410, CellMarque), CD 31 (clone JC70, CellMarque), CD 34 (clone QBEnd / 10, CellMarque). The streptavidin-biotin method (LSAB Kit) was used, diaminobenzidine was used as a chromogen. Unmasking of antigens was performed by heating in citrate buffer (pH = 6.0) for 40 minutes at a temperature of 93 °-95 ° C.

Immunohistochemical study was performed in the Department of Immunohistochemistry of the Belgorod Regional Anatomical Bureau (TS Mukhina). Photorecording was performed on a Mirax Desk scanner (Carl Zeiss Microimaging GMBH, Germany), a Nikon Eclipse Ni microscope, and Nis-Elements BR 4.60.00 software. Statistical calculations were performed using Statistica 10.0.

3. Results and Discussion

The morphological examination of the brain in the first group corresponded to that in healthy animals of both gray and white matter and the hippocampus, in which a three-layer structure was preserved in the proximal large-cell (corresponds to fields CA2, CA3) and distal small-cell regions (equivalent to field CA1). The molecular, pyramidal and marginal layers were well differentiated. It should be noted that non-pyramidal neurons are detected in the molecular layer at a great distance from each other, which are separated by nerve fibers. In the pyramidal layer, neurons of the same name prevailed, and in the marginal zone, mainly nerve fibers and single non-pyramidal neurons. These structural features correspond to the brain morphology of healthy adult rats (33).

As a result of the study, it was found that the recovery of animals in all groups occurred on the 10th day of antibiotic therapy. Histological examination was performed 24 hours after the start of the experiment and on day 11.

In the first group of animals, no significant macro- and microscopic changes were found.

In the second group, macro- and microscopic changes in the brain correspond to the classical description of this process performed by M.A. Skvortsov (34). They manifested themselves in the following changes. Macroscopically, the meninges were dull and full-blooded. Microscopically, revealed a sharp expansion and plethora of the veins of the meninges, lined with endothelium with flat nuclei. Erythrocyte stasis was observed in hemocapillaries and venules. In the area of the intima of arterioles, endotheliocytes are hypertrophied, their large nuclei bulge into the lumen of the vessel.
In the substance of the brain, focal diapedesic hemorrhages, plethora of blood vessels of the microvasculature, pronounced perivascular edema are determined. The marginal glial membrane, in the structure of which the elongated nuclei of astrocytes are visible, separates the brain tissue. Glial cells and a small number of full-blooded capillaries are scattered in the gray matter of the cortex. In the outer granular layer of the cortex, rounded neurons of various shapes with rounded nuclei and nucleoli are not always visible in them. The shape and size of the nuclei significantly predominate over the perikarya. Some of the pyramidal neurons with the phenomena of pycnotic deformation of the nuclei, from the apex of the deformed pyramids and laterally, short dendrites can be traced. Another part of the neurons is sharply hyperchromic, the nuclei in them are not traced (Figure 1).

Figure 1. Neuronal degeneration of CA1 area of hippocampus and cerebral cortex on the 11th day: A – molecular layer, B – pyramidal layer, C – marginal layer, 1 – hyperchromic shrunken neuron; Hematoxylin & eosin. A, B×400.

In the CA1 and CA3 regions of the hippocampus, damaged neurons with karyopyknosis predominate. In addition, rare neurons are found in the form of shadow cells, but with distinct large nucleoli. Typical pyramidal neurons with large nuclei and one or two nucleoli, well-defined dendrites extending from the apex of the pyramids and an axon from the base, are found in smaller numbers, which indicates a high functional load (35-37). The number of glial cells is small here. The vessels of the microvasculature are full-blooded, with perivascular edema (Figure 2).
Thus, on the model of experimental meningitis, it was found that secondary ischemic and toxic lesions of neurons in the cortex and hippocampus accompany typical histological changes in the membranes, and in all sections, there is a pronounced perivascular and pericellular edema, clearly visible when stained with hematoxylin and eosin.

Microscopic examination of the brain of rats of the third group revealed that pyramidal neurons are located in all layers, except for the molecular one. They have a typical structure and different sizes from small (multiforme layer, outer granular and pyramidal layers) and medium (outer and inner granular layers, outer pyramidal layer) to large (inner pyramidal layer) neurons. Moreover, non-pyramidal neurons are found in all layers of the cortex. Therefore, in the upper layers, mainly small horizontal cells are located at a considerable distance from each other. Dense clusters of nerve fibers divide the space between the bodies of these neurons. In the outer granular layer, granular neurons predominate, while in the inner pyramidal layer, a small number of them are revealed. Stellate neurons predominate in the outer pyramidal and inner granular layers. A large number of fusiform neurons are found in the multiforme layer. On preparations in the cytoplasm of neurons, there are accumulations of chromatophilic substances, which manifest itself in the form of varying degrees of chromophilia of the cytoplasm. In small, medium and large cells, these accumulations are distributed in the form of grains throughout the cytoplasm. According to the literature, the amount of basophilic substance in neurons is mainly determined by the intensity of the functional activity of one or another type of nerve cells (33).

According to the degree of chromophilia of the cytoplasm of neurons, the ratio of normochromic (moderate staining of the cytoplasm) and hyperchromic (intense staining of the cytoplasm) neurons on the site of the preparation is visually approximately the same. Hyperchromic shrunken, hypochromic (weak staining) and shadow cells (very weak staining of the cytoplasm) are very rare.
Under experimental exposure, the number of hyperchromic neurons can increase. This can be considered as a hyper function of neurons and their reversible state (33, 38). In the second and third groups, pronounced perivascular and pericellular edema was revealed.

An immunohistochemical study with antibodies to glial fibrillar acidic protein (GFAP) revealed a positive staining of glial elements and their processes, without visible differences from those in the second group. The reaction with antibodies to neuron specific enolase (NSE) and microtubule-associated protein (MAP-2) was negative. The reaction with antibodies to caspase-3 was positive in some neurons (Figure 3). A positive cytoplasmic reaction with antibodies to doublecortin was detected in most neurons of all layers of the brain substance in the form of fine-granular staining. In some neurons, the staining had a clearly pronounced perinuclear character. In single neurons, where the structure of the nucleus was not determined, the staining was diffuse. The reaction with antibodies to the nuclear neuronal protein NeuN revealed an intense cytoplasmic reaction, more pronounced perinuclear (Figure 4). An identical negative reaction with antibodies to CD 31 and CD 34 took place on the 11th day of the experiment in the capillaries of the brain substance.

Figure 3. 11th day. IHC reaction with Caspase-3 (A) and MAP-2 (B) antibodies. A×200, B×100.
Figure 4. 11th day. IHC reaction with Doublecortin (A) and NeuN (B) antibodies: 1 – intensive positive reaction in hyperchromic neurons, 2 – positive reaction in normochromic neurons. A, B×400.

In conclusion: 1. In the first and second groups, a typical structure of an unchanged brain and a typical picture of purulent meningitis were revealed. 2. The microscopic picture, as well as the changes revealed during immunohistochemical study with antibodies to doublecortin and neuronal nuclear protein, characterized by pronounced, mainly cytoplasmic and perinuclear reactions, respectively. Some neurons are positive for caspase-3, which corresponds to the changes characteristic of premature aging.

References


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