

Morphological Changes of the Brain Tissue in Rats with Experimental Model of Ischemic Stroke in the Dynamics of Treatment by Immunobiological Preparation Cryocell-Cryocord

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Investigations of angiogenesis biological characteristics in the post-infarction zone may be of great importance not only for understanding the causes of stroke development, but also for more qualitative treatment planning. And although prognostic and therapeutic principles are only being formed, achievements in understanding the processes of angiogenesis have already been used in clinical practice, and their assessment, perhaps, will be applied in routine clinical activities.

The aim of the investigation was to evaluate the possibilities of using immunobiological preparation Cryocell-Cryocord in the treatment of ischemic stroke on the basis of the findings obtained in the course of studying morphological characteristics of brain tissue and the condition of the rats cerebral capillaries in experiment.

Materials and Methods. 120 white outbred rats were used in our investigation. They were divided into 4 groups, with 30 animals in each: intact rats without focal cerebral ischemia (FCI) and treatment; rats after FCI and with no treatment; rats after FCI with standard treatment; and rats after FCI with standard treatment complemented by Cryocell-Cryocord. A comparative morphometric analysis of the sensorimotor cortex structural components and the newly formed blood vessels in the necrotic area has been carried out during the period of cerebral infarction progression. The ultrastructure has been examined by an electron microscope.

Results. Inclusion of the immunobiological preparation Cryocell-Cryocord into the standard treatment scheme for ischemic stroke has been found to trigger angiogenesis in the infarcted area and to preserve the blood-brain barrier structure, which favored the recovery of the lost functions.

Conclusion. The immunobiological preparation Cryocell-Cryocord has been shown to possess pronounced angioprotective properties and to stimulate endogenous angiogenesis, which allows it to be included into the treatment scheme for ischemic stroke.

Key words: angiogenesis; ischemic stroke; reparation; endothelial cells; necrosis; hypoxia.

In spite of numerous fundamental and clinical investigations the problem of prevention and management of ischemic stroke is far from being solved. Different groups of medications (thrombolytics, anti-inflammatory drugs, nootropics, antioxidants) and physiotherapeutic procedures in various combinations are used to treat the condition, but all the suggested methods fail so far to give radical solution of the problem. Therefore,

principally new approaches are being searched for the conservative treatment of the disease [1].

Rather promising may be methods of targeted tissue regeneration and therapeutic angiogenesis, which aim to activate compensatory resources of the damaged cells, tissues, vascular system, to stimulate the mechanisms of restoration and regeneration, to replace the lost structures and functions of the organism, organ or tissue [2, 3].

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This medical technology has already been used in treating various destructive processes of parenchymal organs (hepatic cirrhosis); during restorative period in viral hepatitis, chronic hepatic insufficiency; for detoxication; in congenital and acquired immunodeficient conditions, anemias of different origin, infertility, thymus hyperplasia, pancytopenia; in the complex treatment of inflammatory processes in surgical and dental practice; in therapy of insulin-dependent diabetes and its complications; in hemopoiesis impairment after chemo- and radiation therapy. Information on its application for treating patients with ischemic stroke is far too limited [4].

This technology implies receiving a number of balanced biologically active natural compounds capable to influence various aspects of metabolism of the whole organism. A preparation of human cryopreserved cord blood serum Cryocell-Cryocord, containing such biologically active compounds as hemopoietins, adaptogens, opioid peptides, enzymes, a complex of reproductive immunomodulators, vitamins was used by us to treat ischemic stroke.

Understanding the role of local and general factors in the origin and progression of ischemic stroke, i.e. changes of the cytokine status, rheological blood properties and direct vascular network damage in the necrosis area, as well as experimentally established protective efficacy of Cryocell-Cryocord, primarily due to its antihypoxic and antioxidative properties, ability to stimulate reparative processes in the affected area has spurred us to study the effect of the preparation on the process of angiogenesis in the rats with the modeled focal cerebral ischemia (FCI).

The aim of the investigation was to study morphological features of the brain tissue and the condition of cerebral capillaries of the rats with an experimental model of ischemic stroke during their treatment with immunobiological preparation Cryocell-Cryocord in order to evaluate its angioprotective and angiogenesis-stimulating properties.

Materials and Methods. Investigations were conducted on 120 out-bred white male rats weighing 200 ± 20 g. The work was performed in accordance with ethical principles established by European Convention for the Protection of Vertebrata used for Experimental and other Scientific Purposes (the Convention was passed in Strasburg, March, 18, 1986, adopted in Strasburg, June, 15, 2006) and recommendations of the Bioethics Committee of Medical Institute of Sumy State University.

All animals were divided into 4 groups: group 1, intact rats without ischemia and treatment; group 2, animals after FCI without treatment; group 3, animals with standard treatment; group 4, animals after FCI with standard treatment complemented by Cryocell-Cryocord preparation.

All operative interventions were performed under intraperitoneal anesthesia in the dosage of 20 mg/kg of sodium thiopental. FCI was modeled by injecting barium

sulfate suspension in the sterile saline solution in the proportion 1:3 into the right carotid artery through the incision in the soft tissues of the neck [5]. The quantity of suspension introduced intra-arterially amounted to 0.1–0.3 ml.

The standard treatment included introduction of 40 mg/kg 25% solution of magnesium sulfate and 15 mg/kg of citicoline solution. In group 4 it was complemented by 100 μ l of immunobiological preparation Cryocell-Cryocord, manufactured at the Interdepartmental Research Center of Cryobiology and Cryomedicine (Kharkov, Ukraine). This is a cryoconserved cell-free preparation from the human placental blood serum, containing biologically active substances — monokines, interleukines, interferons; hormones — steroidal, estrogens, gestogens, testosterone, progesterone and others; a complex of reproductive immunomodulators, growth factors, hemopoietins, adaptogens, enzymes, microelements, vitamins.

The preparation was measured according to the content of the total protein and α -fetoprotein: 1 ml contains 21.0 ± 0.8 mg of the total protein and $2,100 \pm 600$ ME of α -fetoprotein. Preparations were given intraperitoneally to the survived rats 12 h after the beginning of FCI modeling, and on day 2, 3, and 4. Psychoneurological status of the animals was controlled daily. The material for morphologic investigation was taken 7 days after FCI modeling.

Brains of the experimental animals were fixed for morphological examinations by transcatheter perfusion of the mixed solutions of 4% paraformaldehyde, 1% glutaraldehyde, 5% saccharose in 0.1 M phosphate buffer (pH=7.4) during 15–20 min under 90 mm Hg. After material fixation light optical (imbedded in paraffin) and electron microscopy specimens were prepared.

Using stereotaxic atlas of the mature rat brain, a region of sensorimotor cortex (SMC) (Fpa and Fpp fields) was outlined for electron microscopic examination [5]. Ultrathin sections (70–100 nm) of all SMC layers were used.

Ultrastructure was studied using electron microscope PEM-125K at accelerating voltage of 75 kV, provided with the system of image capturing and analyzing SAI-01A (SELMI, Ukraine), based on CCD-camera DX-2 and a software package (KAPPA, Germany).

Histological and physiological approaches approach served as a methodological basis of morphological investigation of the brain SMC: great attention was paid to the comparative morphometrical analysis of the structural components of such a complex multilevel system, as the dynamically progressing SMC in the post-ischemic period is [6, 7].

Results and Discussion. In the post-ischemic period impairments of hemostasis were found to be structurally present at all levels of the SMC microvascular network. These structural changes involved blood formed elements and plasma, microvessel walls (endothelium,

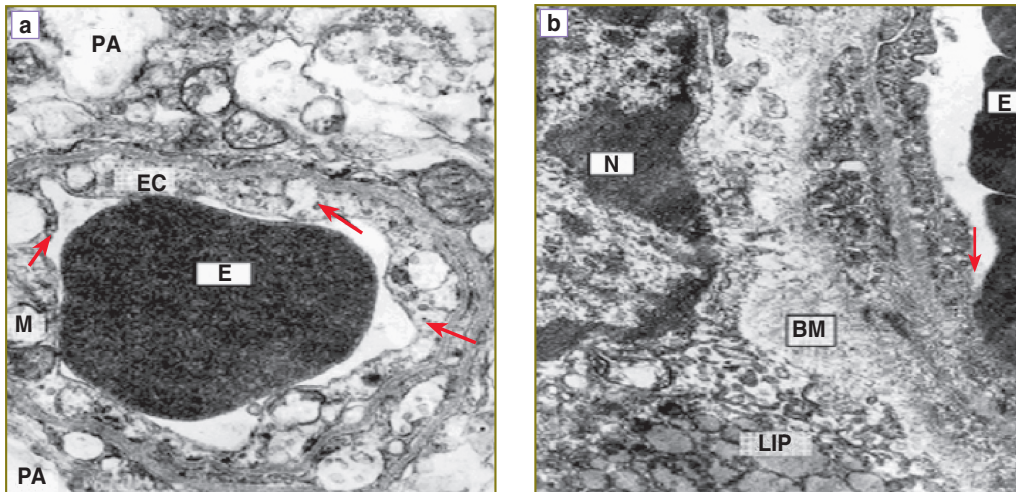


Figure 1. Capillary of layer III of the white rat sensorimotor cortex on day 7 after focal cerebral ischemia without treatment: (a) change of endothelial cells (EC), bright type, mitochondrial (M) swelling and destruction, vacuolization of cytoplasm, large number of endocytotic vesicles (arrows), erythrocyte (E) adhesion to the endothelial cells surface, edema of the perivascular processes of astrocytes (PA); $\times 15,300$; (b) aggregation and deformation of E, their adhesion to the endothelial cells surface (arrow), swelling and destruction of the basement membrane (BM), accumulation of lipids (LIP) in pericyte cytoplasm, N: pericyte nucleus; $\times 19,800$

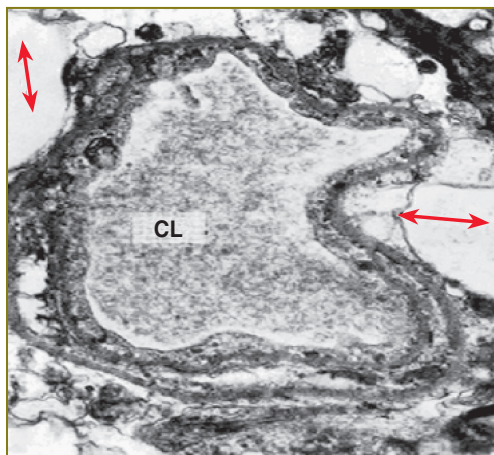


Figure 2. Capillary of layer III of the white rat sensorimotor cortex on day 7 after focal cerebral ischemia without treatment. Cerebral capillary with an increased protein content, a marked edema of astrocyte perivascular processes (double arrows), CL: capillary lumen; $\times 17,500$

basal membrane (BM), pericytes), and astroglia vascular legs as well. Blood flow arrest caused adhesion of erythrocytes to the luminal surface of endothelial cells (EC) (Figure 1).

The most variable alterations occurred in the cerebral capillary EC. Ultrastructural manifestations of their reactive changes varied from insufficient dystrophic to the marked necrobiotic.

Mitochondrial swelling, appearance of vacuoles, destruction of polyribosomal ribosomal rosettes, distribution of ribosomes, their separation from the granular reticulum membrane, thickening and

microclasmatosis of EC, appearance of long cytoplasmatic processes, varicose lesions of endothelium, alteration of pinocytotic vesicle content, reduction of intracellular connections, breaking of slit-like contacts and BM thickening were referred to insignificant, initial signs of reactive changes (Figure 2).

The data obtained show, that in the post-ischemic period aggregation properties of blood cells and their interrelations with EC surface change significantly, part of them are subjected to complete or partial destruction accompanied by the release of biologically active substances into the bloodstream. When a vessel wall is affected, an isolating function of EC is lost, subendothelial layer becomes exposed, BM collagen fibers are bare, promoting Hageman factor activation, change of the charge and zeta-potential [8, 9].

Destructive alterations in the cerebral capillaries combine with stimulation of intact EC, resulting in the increase of both relaxants and constrictors [10, 11]. Enhancement of the vascular permeability is structurally manifested by EC decrease; rearrangement of their cytoskeleton and contacts; damage of epithelium with retraction, lysis and detachment of endothelium or endothelium detachment without lysis [12–14].

The signs of blood-brain barrier dysfunction were also noted: fusion of separate BM areas, swelling of EC and pericytes, edema and destruction of perivascular glia.

The analysis of the structural and vascular impairments in the rat brains with the modeled ischemic stroke shows, that only normalization of all main components, providing adequate homeostasis (the condition of vascular wall, blood cells and plasma) will enable to minimize aftereffect of the secondary microcirculation disorder.

Unfortunately, traditional methods of treatment fail to provide anticipated favorable effect, and this urges to search new therapeutic approaches. It was for the purpose of stimulation of angiogenesis and normalization of metabolic processes in the infarction area that the classic FCI treatment was complemented by immunobiological preparation Cryocell-Cryocord, containing various biologically active substances, growth factors and hormones.

Findings of our investigation have convincingly shown, that addition of Cryocell-Cryocord to the classic scheme of FCI treatment resulted in statistically significant decrease of the perivascular edema area by 21.4%, whereas in the group of animals receiving only standard treatment this parameter reduced only by 12.7% ($p < 0.01$), and in the group without any treatment by 11.3% ($p < 0.01$). Besides, in group 4 adhesion of formed blood elements to vascular endothelium decreased, EC edema dissipated, resistance of erythrocytes to hemolysis increased, diminishing the signs of ischemic damage. Similar changes were noted in other examined groups, but they were significantly less marked compared to the group of animals receiving additionally Cryocell-Cryocord.

On day 7 there was registered an increase of the elements of newly forming cerebral capillaries by 34.7% ($p < 0.01$) in group 4 relative to the intact animals, and by 22.3 and 18.8% in groups 2 and 3, respectively.

Application of magnesium sulfate and citicoline solutions together with Cryocell-Cryocord stimulated restoration of the damaged capillary ultrastructure and growth of new capillaries on the basis of the existing ones (Figure 3).

It was especially characteristic for the capillaries, in which BM and pericytes preserved. On this framework a new layer of endothelium, replacing the dead EC, was formed. As a rule, a new capillary had a split BM.

When Cryocell-Cryocord was used, such manifestations and signs of exhausted reparative capabilities as reduction of the working vessel lumen, accumulation of the remaining corpuscles in the EC cytoplasm, proliferation of perivascular astroglia, accumulation of lipids, secondary lysosomes, fibrillar structures in it, and EC atrophy diminished or even disappeared. Upon the whole, the ultrastructure of the vessel wall looked more preserved and damage to BM was not so deep.

In the neogenic vessels in the material, taken from the animals of group 4, the perivascular space did not differ from the normal. However, a significant number of moderately edematous astrocyte legs with a small quantity organelles were noted. Vessel lumens were, as a rule, clear, filled with plasma (Figure 4).

The described changes are likely to be adaptive mechanisms in acute FCI. Acute hypoxia and discharge of a great quantity of mediators in the ischemic zone are powerful stimulators of angiogenesis. Activation

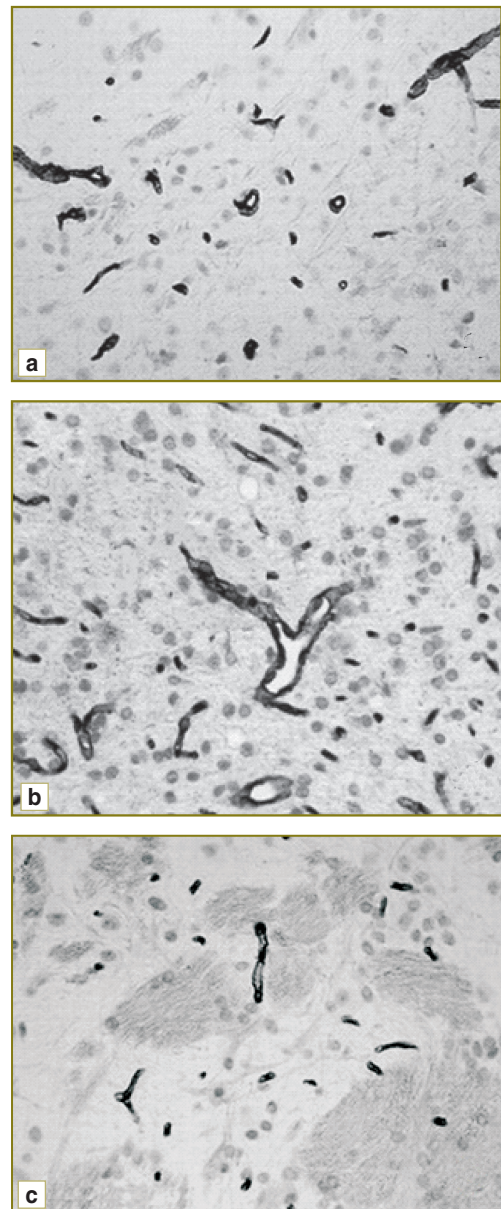


Figure 3. Layer III of the white rat sensorimotor cortex on day 7 after focal cerebral ischemia with standard treatment (a), with introduction of Cryocell-Cryocord into the treatment scheme (b), a similar layer on the contralateral hemisphere (c) is given for comparison. On Figure (a) and (b) increase of cerebral capillary dimensions and vascular density compared to contralateral hemisphere (c) is noted; stained with hematoxylin and eosin, $\times 40$

of endogenous angiogenesis in the brain by a series of biologically active substances, contained in the immunobiological preparation Cryocell-Cryocord, may also be one of the mechanisms of these changes, as its application proved to lead to a significantly more active stimulation of angiogenesis in contrast to the group of animals, receiving only standard treatment. The encouraging results, obtained on animals, may become an incentive to further investigation of the effect of Cryocell-Cryocord on patients with ischemic stroke.

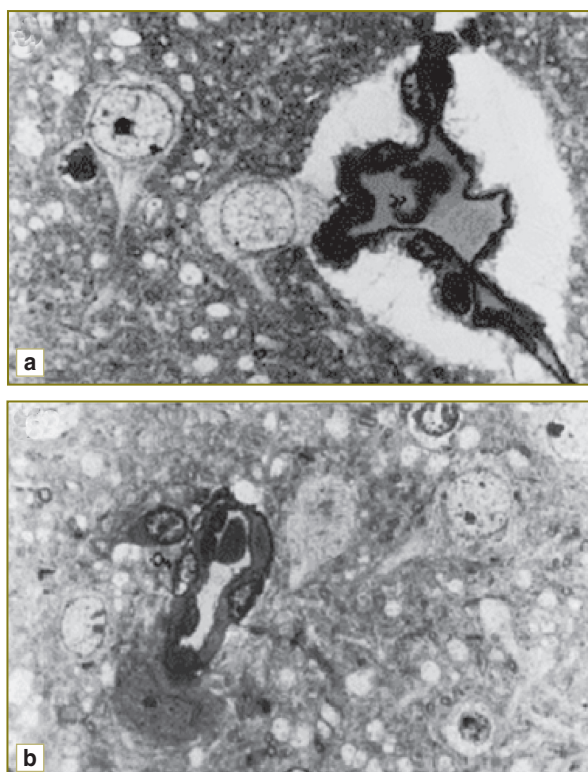


Figure 4. Cortex of the cerebral hemispheres of the rats without treatment from group 2 (a) and animals, which were given magnesium sulfate and citicoline solutions together with Cryocell-Cryocord (b); stained with hematoxylin and eosin, $\times 120$

Conclusion. Using a model of experimental focal cerebral ischemia in the course of treatment complemented with immunobiological preparation Cryocell-Cryocord, it has been established that the preparation shows marked angioprotective properties, prevents stasis of capillaries, preserves the structure of blood-brain barrier, activates angiogenesis in perinecrotic zone by increasing the number of functioning capillaries and exchange surface of the capillary bed. Expressiveness of the vascular reaction on experimental day 7, a dense arrangement of capillaries and availability of numerous anastomoses indicate to the activation of collateral circulation in perinecrotic zone in the rats, receiving complementary Cryocell-Cryocord.

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