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EFFECT OF VEGF ON VESSEL DISTRIBUTION IN PATIENTS WITH STROKE

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Abstract *VEGF-A is considered a key factor initiating neoangiogenesis after ischemic stroke, but its relationship to vascular changes in human brain tissue remains poorly understood.*

The aim of the study was to evaluate VEGF-A expression and vascular density in the frontal cortex at various time points after an ischemic stroke. The study was conducted using autopsy material from 51 patients with cerebral infarction (ICD-10:I63.3/I63.4). Histological analysis and immunohistochemical staining with antibodies against CD31 and VEGF-A were performed.

Vascular density and the ratio of VEGF-A-positive endothelial and neuronal cells were quantified in the infarct nucleus and in the penumbra. Statistical analysis was performed using the Mann-Whitney U–test. A significant decrease in vascular density was found in the heart attack site. An increase in VEGF-A levels and an increase in vascular density in the penumbra reflect an early compensatory angiogenic reaction. The presence of VEGF-A in neurons indicates a potential cytoprotective role in ischemia.

Keywords: *stroke, angiogenesis, growth factors, immunohistochemistry, blood vessels*

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ВЛИЯНИЕ VEGF НА РАСПРЕДЕЛЕНИЕ КРОВЕНОСНЫХ СОСУДОВ У ПАЦИЕНТОВ С ИШЕМИЧЕСКИМ ИНСУЛЬТОМ

Аннотация. *VEGF-A считается ключевым фактором, инициирующим неоангиогенез после ишемического инсульта, однако его связь с сосудистыми изменениями в ткани головного мозга человека остается недостаточно изученной.*

Целью исследования стала оценка экспрессии VEGF-A и плотности сосудов в лобной коре головного мозга в различные моменты времени после ишемического инсульта.

Исследование проводилось с использованием аутопсийного материала 51 пациента с инфарктом головного мозга (МКБ-10: I63.3/I63.4). Были проведены гистологический анализ и иммуногистохимическое окрашивание антителами против CD31 и VEGF-A. Плотность сосудов и соотношение VEGF-A-позитивных эндотелиальных и нейрональных клеток были количественно оценены в ядре инфаркта и в полутени. Статистический анализ проводился с использованием U–критерия Манна-Уитни.

В очаге инфаркта было обнаружено значительное снижение плотности сосудов. Повышение уровня VEGF-A и увеличение плотности сосудов в полутени отражают раннюю компенсаторную ангиогенную реакцию. Присутствие VEGF-A в нейронах указывает на потенциальную цитопротекторную роль при ишемии.

Ключевые слова: *инсульт, ангиогенез, факторы роста, иммуногистохимия, кровеносные сосуды*

Introduction. Ischemic stroke remains a leading cause of mortality and disability worldwide [1]. The nature of the vascular response significantly influences functional outcomes in the post-ischemic period. However, current therapeutic strategies have not yet achieved substantial success in reducing stroke incidence. Despite extensive research, further morphological studies are needed to uncover new mechanisms of stroke pathogenesis, enabling the development of personalized, targeted therapies.

Modern treatment approaches primarily aim to preserve the histoarchitecture of the penumbra [2]. Still, none have proven effective in significantly reducing the

volume of necrotic neural tissue. It appears especially relevant to investigate neovascularization which capable of protecting penumbral neurons and limiting infarct expansion [3]. Key regulators of neovasculogenesis include members of the vascular endothelial growth factor (VEGF) family, particularly VEGF-A and VEGF-B [4]. Yet, the correlation between VEGF levels and vascular density remains to be conclusively demonstrated. Furthermore, comparative analysis of vascular distribution across ischemic brain regions is of high interest.

Although numerous studies have addressed vascular responses to cerebral ischemia [3], most are either experimental or based on small, heterogeneous patient samples [5]. Therefore, the direct examination of autopsy brain tissue from a large patient cohort offers valuable insights. Equally important is the attempt to correlate VEGF-A concentration in brain homogenates with microvascular density using modern histological and immunohistochemical methods.

The aim of the study – immunohistochemical assessment of neoangiogenesis and blood vessel distribution in the acute phase of cerebral cortical infarction.

Materials and Methods. Archival autopsy material for this study was obtained from patients (mean age 45–59 years) within no more than 6 hours after the official confirmation of death.

Group I (n = 51) consisted of paraffin-embedded brain cortex samples (frontal lobe) obtained from patients with a confirmed diagnosis of cerebral infarction (ICD-10: I63.3 / I63.4), verified by clinical and anamnestic data, neuroimaging techniques (CT / MRI of the brain), and postmortem examination findings. The infarction was classified as cardioembolic or atherothrombotic according to the TOAST criteria. The time from the known or estimated onset of symptoms to death did not exceed 7 days.

Group II (n = 10) included autopsy material from patients who died due to extracranial causes. These brain specimens were considered morphologically intact.

Exclusion criteria for both groups included: hemorrhagic or mixed-type stroke of any localization and etiology, traumatic brain injury, coexisting CNS pathology, hematologic, autoimmune, and/or systemic oncological diseases, acute bacterial or viral infections, chronic alcohol abuse, or damaged autopsy material.

Histological examination was performed using standard protocols. Neuronal count was assessed using hematoxylin and eosin staining. Immunohistochemical analysis of VEGF-A and CD31 expression was conducted using specific primary antibodies (monoclonal anti-CD31 – ThermoFisher, Clone JC70A; polyclonal anti-VEGF-A – Sigma-Aldrich, Cat. No. ABS82). For detection of secondary antibodies, the HiDef Detection™ HRP Polymer system (Cell Marque, USA) was used, along with anti-mouse/rabbit IgG, horseradish peroxidase (HRP), and DAB substrate. Cell nuclei were counterstained with Mayer's hematoxylin. CD31 was used to visualize and count blood vessels, while VEGF-A expression (as a percentage) was evaluated in neurons and vascular endothelium separately in the infarct core and penumbra.

The data were analyzed using SPSS 12 for Windows (IBM Analytics, USA). Results were expressed as mean ± standard deviation (SD). Paired comparisons

between groups were performed using the Mann–Whitney U test with Bonferroni correction. A p-value of ≤ 0.05 was considered statistically significant.

Results. In cortical brain samples from Group I patients, typical morphological features of cortical infarction were identified. These included a pannecrotic zone characterized by a significantly reduced number of neurons compared to the control group (4.1 ± 0.2 vs 18.8 ± 0.9; $p < 0.001$), eosinophilic cytoplasm in neuronal perikarya, and nuclear pyknosis. This was accompanied by local microcirculatory disturbances in the infarct core, including venous congestion, perivascular edema, erythrocyte aggregation, and stasis. In contrast, changes in the penumbra were less pronounced (14.7 ± 0.7; $p < 0.001$) and were accompanied by moderate infiltration of lymphocytes and polymorphonuclear cells.

Immunohistochemical analysis of CD31 revealed that vascular density in the cerebral cortex following ischemic stroke depended on both the time elapsed since stroke onset and the specific brain region. In all cases, there was a significant reduction in vessel count within the infarct area compared to controls (0.8 ± 0.1 vs 4.6 ± 0.2; $p < 0.001$). In the penumbra of patients who died within the first 24 hours post-stroke (36% of cases), vascular reduction was also observed (1.1 ± 0.1; $p < 0.001$). Conversely, in patients who died two or more days after stroke onset (64%), vascular density in the penumbra increased (4.3 ± 0.2; $p < 0.001$), due to the proliferation of small arterioles, confirmed by strong membranous CD31 expression in the endothelium.

Immunohistochemical analysis of the angiogenic factor VEGF-A showed heterogeneous endothelial expression depending on vessel caliber. In Group I, the most intense staining was observed in small vessels, whereas in large arteries, VEGF-A expression was limited to weak cytoplasmic staining in individual endothelial cells. In the infarct core, VEGF-A was detected only in a few preserved vessels ($5.4 \pm 0.2\%$; $p < 0.001$). In contrast, the number of positively stained vessels in the penumbra significantly exceeded that in the control group ($41.8 \pm 2.0\%$ vs $14.2 \pm 0.7\%$; $p < 0.001$).

Particular attention should be paid to VEGF-A expression in cortical neurons after stroke. In patients who died 2–3 days after stroke onset (58% of cases), a characteristic granular cytoplasmic VEGF-A staining was observed in approximately half of the neurons in the penumbra compared to controls ($41.5 \pm 2.0\%$ vs $2.3 \pm 0.1\%$; $p < 0.001$). A small number of preserved neurons in the central infarct zone also showed positive staining ($11.3 \pm 0.5\%$; $p < 0.001$).

Conclusion. An increase in VEGF production, along with the proliferation of small vessels, is considered an early key mechanism in the activation of compensatory neoangiogenesis aimed at supporting neuronal metabolism in the penumbra. A cytoprotective effect of this factor in neurons, as well as its potential role in neurogenesis, cannot be excluded. In contrast, ischemic damage to the cortex is accompanied by multifactorial endothelial cell death, likely associated with disruption of the blood-brain barrier and microcirculation, enhanced oxidative stress, and a pronounced inflammatory response. However, the relationship between VEGF

and these pathophysiological mechanisms of ischemic stroke remains to be fully elucidated.

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