Cortisol as a cerebral cortex neurons apoptosis regulator in acute phase of ischemic stroke (clinical and pathological study)

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In response to ischemic stroke (IS) a natural activation of the stress-realizing system occurs. The features of this activation influence the outcome of the acute period and the prognosis of recovery and can be adjusted. At the same time, the role of the stress-realizing system in the pathogenesis of IS is still unexplored.

Objective: *to investigate the effect of peripheral blood cortisol concentration on the regulation of apoptosis of neurons of the cerebral cortex in the acute phase of IS.*

Material and methods. *A prospective clinical and pathological study was performed. It included 9 patients with IS in the left middle cerebral artery territory who were admitted to hospital and died in the hyperacute phase of IS and had no infectious complications, allergic reactions or oncological diseases and who did not undergo thrombolysis. The cerebral cortex was examined. Neuron-specific enolase (NSE), protein 53 (p53), caspase 3, caspase 8, Fas receptor (CD95), and Fas apoptotic inhibitory molecule 2 (FAIM2) were determined on the slices using an indirect immunoperoxidase immunohistochemical staining method. A total of 567 microscopic fields were analysed for the group of patients with IS and 63 fields for the control group (three people). Before death, the blood concentrations of sFas, sFasL, cortisol, adrenocorticotropic hormone, adrenaline and norepinephrine were determined by enzyme immunoassay (the control group consisted of 28 people).*

Results. *Significant correlation was found between the proportion of casp3-positive neurons and the concentration of cortisol in peripheral blood in zones 2 ($r=0.263$; $p<0.01$) and 3 ($r=0.383$; $p<0.01$). In the 2nd zone, significant negative correlation was found with the concentrations of sFas ($r=-0.177$; $p<0.05$) and sFasL ($r=-0.164$; $p<0.05$); in the 3rd zone, significant positive correlation was found with the ratio of the concentrations of sFasL and sFas ($r=0.240$; $p<0.01$). The proportion of Fas-positive neurons in the cerebral cortex correlated significantly with the concentration of the soluble form of this molecule (for the 1st zone – $r=0.222$, for the 2nd zone – $r=0.438$, for the 3rd zone – $r=0.289$; $p<0.01$) and the ratio of the concentrations of sFasL and sFas (respectively: $r=0.231$, $r=0.266$ and $r=0.281$; $p<0.01$) in the peripheral blood.*

Conclusion. *Peripheral blood cortisol concentration is a factor that determines the regulation of apoptosis of neurons in the cerebral cortex in the acute phase of IS.*

Keywords: *apoptosis; stroke; cortisol; adrenocorticotropic hormone; stress; adrenaline; norepinephrine.*
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Ischemic stroke (IS) is a common disease that has significant medical, social, and economic consequences for society [1]. It has been established that one of the main mechanisms of neuronal death in the brain during all stages of its development (acute, subacute, and recovery) is apoptosis – a complex energy-dependent process of regulated cell destruction [2]. During IS, in the neurons of the brain, mechanisms of caspase-independent apoptosis as well as caspase-dependent internal and external pathways can be realized [3]. In the regulation of cell death, the transcription factor p53 plays a significant role in pro-apoptotic processes. It is associated with the initiation of caspase-independent and internal caspase-dependent pathways of apoptosis [4]. Earlier, we published data on changes in the morphological structure of the brain cortex after IS, and on the features of p53 expression by neurons and astrocytes [5]. This work allowed us to formulate new questions and served as a starting point for the presented study. Caspase-3 (casp3) is considered a universal indicator of apoptotic cell activity, and its activation plays a significant role in neuronal apoptosis and is considered a terminal event preceding cell death [6]. The extrinsic pathway of caspase-3 activation is caspase-8-dependent and is initiated by the binding of death ligands to their receptors. One such example is the ligand and receptor of the Fas system. There are soluble forms of its ligand (sFasL) and receptor (sFas), which are mainly formed as a result of shedding and alternative splicing. Membrane receptors can be activated by both soluble and membrane-bound ligands. In turn, soluble forms of receptors

can bind and neutralize ligands [7]. Several studies have highlighted the significance of the Fas/FasL system in the pathogenesis of the acute phase and the development of long-term consequences of IS: the volume of nervous tissue damage in various pathologies has been associated with the concentration of soluble Fas factors in the blood and the severity of the condition. In other studies, an increase in the levels of Fas and FasL has been noted in compromised areas of the brain during the modeling of various neurological disorders, including IS, in animals [8]. At the same time, the initiation of apoptosis and activation of apoptosis-inducing cascades in a cell do not always mean that the cell will die at all or die through apoptosis implementation. The apoptotic signal can have effects that contribute to the restoration and adaptation of the cell population to the conditions that triggered apoptosis mechanisms [9]. A family of Fas-inhibitory molecules has been described, among which the molecule Faim2 is of particular interest in the context of the raised issue. Increased expression of Faim2 leads to a decrease in Fas-associated apoptosis of neurons in various pathological conditions [10].

It is also necessary to note that under conditions of energy deficit, apoptosis cannot be fully realized, causing the cell to switch to another mode of death that requires less energy and may be significant for worsening the clinical manifestations of stroke and the severity of the patient's condition [11]. Based on the above, the regulation of apoptosis and associated processes in brain neurons during the development of IS appears to be significant. This requires studying the influence of individual factors on its implementation and the clinical picture of the disease. Earlier, we obtained data on the influence of concentration of stress-system hormones on the outcome of the acute phase of IS and on morphological changes in the brain of patients after IS [5, 12], which indicated that cortisol concentration in peripheral blood could be one of such factors. It has been shown that cortisol concentration influences the implementation of apoptosis by neurons in cell culture and animal models [13]. Cortisol concentration in peripheral blood increases with the development of depression, which is associated with the activation of neuronal death processes in the brain [14]. Additionally, nearly 40% of stroke survivors develop depression that significantly worsens their recovery prognosis [15]. At the same time, several authors consider IS to be a stressor, triggering a predictable activation of the stress system. The characteristics of its activation influence the outcome of the acute phase and the prognosis of the recovery period [16]. Clinical methods exist to regulate the intensity of the stress system response, as well as the predominant axis (sympathoadrenal, considered protective, or hypothalamic-pituitary-adrenal, with its main mediator cortisol, which worsens the prognosis) [17, 18].

The aim: of this study is to investigate the influence of peripheral blood cortisol concentration on the regulation of apoptosis in the neurons of the cerebral cortex during the acute period of IS (clinical and pathological study).

Material and methods. The material for the study consisted of tissue samples from the cerebral cortex of nine patients (five males and four females) who experienced their first IS in the territory of the left middle cerebral artery. These patients were admitted to a specialized hospital during the acute period and died within 7 days of hospitalization. They did not undergo reperfusion therapy, and at the time of hospitalization they did not have any allergic reactions, infectious, oncological, or autoimmune diseases. Tissue samples from patients who died after IS were obtained from the biobank of the Pathological Anatomy Department of Clinical Hospital No. 36 named after F.I. Inozemtsev. Control group samples (three individuals) were obtained from the biobank of the Bureau of Forensic Medical Examination of the Moscow Department of Health in the form of paraffin blocks. The conduct of the scientific study did not affect the fact of conducting pathological anatomical research, did not involve changing its protocol, and consisted of additional histological and immunohistochemical examination of samples provided from the biobanks.

Three zones of the cerebral cortex were investigated: Zone 1 was adjacent directly to the focus of necrotic tissue, Zone 2 was located 4–7 cm away from the previous zone, and Zone 3 was in the contralateral hemisphere and symmetrical to the focus of the IS. In the control group, one sample of cerebral cortex tissue from the area of the left middle cerebral artery blood supply was examined. Sections with a thickness of 5 micrometers were prepared using a rotary microtome Leica RM2125RT (Leica, Germany). The obtained sections were studied using a light microscope AxioScope A1 (Carl Zeiss, Germany) equipped with a Canon Power Shot digital camera, using AxioVision LE software (Carl Zeiss, Germany). Visual assessment and morphometric analysis of the cerebral cortex were performed on digital photographs obtained with the above-mentioned equipment (objective ×40, aperture 0.9) with an image size of 1300×1030 pixels, a real size of the captured area of 220×174 micrometers (38,280 square micrometers). Seven random fields of view were examined for each of the three sections of each of the three zones in each of the nine patients in the IS group (189 fields of view for each of the three zones). In total, 567 fields of view were processed for the IS patient group. In the control group, seven fields of view were processed for each of the three sections of each of the three individuals (n=63).

Tissue sections were subjected to indirect immunoperoxidase immunohistochemical staining to detect neuron-specific enolase (NSE), protein 53 (p53), caspase-3 (casp3), caspase-8 (casp8), Fas receptor (CD95), Fas ligand (CD178), and Fas apoptotic inhibitory molecule 2 (FAIM2). Monoclonal antibodies specific to these human proteins (Vision BioSystems Novocastra, UK) were used for immunophenotyping, along with the Peroxidase Detection System for Novocastra (Leica Microsystems, Germany), which includes secondary universal biotinylated antibodies and streptavidin-peroxidase complex. Visualization of the reaction was achieved using DAB chromogen. Hematoxylin Mayer was used for background staining of the sections.

Upon admission to the hospital, the severity of neurological deficit in all the aforementioned patients was assessed using the National Institutes of Health Stroke Scale (NIHSS). Additionally, standard test systems and a Multiscan EX multichannel spectrophotometer (Labsystems, Finland) were used for enzyme-linked immunosorbent assay (ELISA) to determine the levels of sFas, sFasL, cortisol, adrenocorticotropic hormone (ACTH), adrenaline, and noradrenaline in peripheral blood. Participation in the study did not alter the plan of diagnostic and therapeutic measures: all patients received medical care in accordance with the standard of care for stroke patients.

The study complied with the principles of the Helsinki Declaration of the World Medical Association (2000) and was approved by the Interuniversity Ethics Committee (extract from the meeting protocol dated 26.09.2019 No. 08-19). All participants were included in the study after signing an informed consent form approved by the ethics committee. The control group for assessing laboratory parameters consisted of 28 relatively healthy individuals matched by age with the study group (15 males and 13 females).

The data were statistically processed using Statistica 6.0 software (StatSoft, USA). To test the hypothesis regarding the distribution law of the data, the Pearson criterion, chi-square criterion, and Kolmogorov–Smirnov criterion were applied. In case of non-confirmation of normality of the distribution, the median was indicated for the sample, with the lower and upper quartiles provided in parentheses (Me [25^{th}; 75^{th} percentiles]). Non-parametric methods were used for working with such samples. If the normality of the distribution was confirmed, quantitative data were presented as the mean (M) ± standard deviation (SD). Parametric methods of analysis were used for comparing the means. The significance level of the difference between two samples (p-level) was assessed using the Student's t-test. Differences were considered statistically significant at $p<0.05$.

Results. The average age of the patients included in the study was 66.9±9.9 years, while in the control group, it was 63.2±8.4 years. The average score on the NIHSS scale upon admission to the hospital for patients with ischemic stroke was 14.1±3.3. The main cardiovascular diseases in all patients included in the main research group were arterial hypertension and atherosclerosis. The primary cause of death among the patients included in the study was the development of pulmonary artery thromboembolism.

The table presents the concentrations of the investigated parameters in peripheral blood in the group of patients with ischemic stroke (IS) and the control group. Significant differences were found in the following parameters: sFasL, cortisol, ACTH ($p<0.01$).

Upon staining sections of the brain cortex from patients who died after an ischemic stroke with hematoxylin and eosin, and performing immunohistochemical reactions to detect NSE, we revealed changes, the nature of which depended on the distance of the examined zone from the ischemic focus. In Zone 1, adjacent to the area of necrotic changes, and in Zone 2, located 5–7 cm away from the first, the cytoarchitecture was significantly altered, with a loss of differentiated layers of the brain cortex.

In Zone 3 and in the control group, the brain cortex had a characteristic organization. Both in the ipsilateral (Zones 1 and 2) and contralateral to the IS focus (Zone 3) hemispheres, diffuse "failure" of areas of the brain cortex was evident. The severity of these changes in the ipsilateral hemisphere was greater and most pronounced in Zone 1.

In all areas of investigation, using immunohistochemical staining for detecting NSE, routine hematoxylin and eosin staining methods, Nissl staining, and light microscopy, morphological changes characteristic of ischemic damage were identified: in Zone 1, 90 [83.3; 94.7] % of neurons were affected, in Zone 2 – 74.2 [69; 78.1] %, and in Zone 3, the proportion of damaged neurons was 13.9 [9.8; 18.6] %, which also significantly differed from the control values (12.1 [8.3; 15.4] %). In the necrotic focus and the adjacent Zone 1, leukocytic infiltration was observed.

Changes in regional blood flow were detected in all investigated areas, including venous hyperemia, stasis, erythrocyte aggregation, perivascular edema, and endotheliocyte swelling.

The implementation of apoptosis mechanisms in neurons in the histological sections of the cerebral cortex of patients who died after ischemic stroke (IS) and in the control group was assessed based on the presence of proteins p53, casp8, casp3, which were detected using immunohistochemical analysis (see Figure, *a–c*). Immunohistochemical reaction for determining NSE was performed on duplicate sections.

In the control samples, the proportion of p53-positive neurons was 20.4 [14.8; 25] %. Significant differences were observed in samples from all zones after cerebral ischemia compared to the control group ($p<0.01$). In Zone 1, the highest representation of p53-positive neurons was noted, with their proportion being 85 [80; 90.9] %, which was significantly higher ($p<0.01$) than in Zone 2 (33.3 [27.6; 40.5] %). In Zone 3, the proportion of p53-positive neurons was significantly lower ($p<0.01$) than in the second zone: 26.1 [18.2; 33.3] %.

The maximum percentage of neurons expressing casp3 (88.2 [81.3; 94.1] %) was observed in samples from Zone 1; as the distance from the ischemic focus increased, their percentage significantly decreased to 50 [41.9; 55.6] % in Zone 2 and to 39.3 [34; 44.4] % in Zone 3. In all zones of the cerebral cortex samples after cerebral ischemia, the differences from the control group's indicators (19.7 [13.4; 26.3] %) were significant ($p<0.01$).

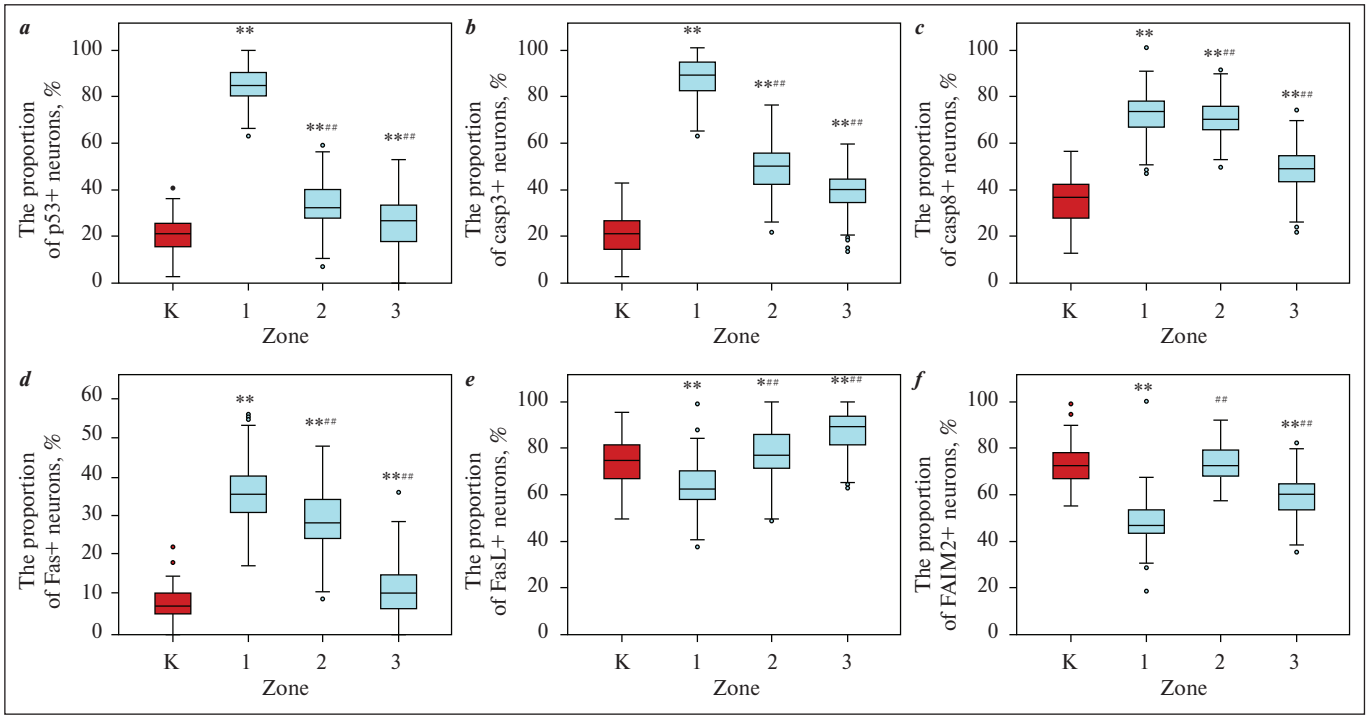
Casp8 was detected in 73.1 [66.7; 78.6] % of neurons in samples from Zone 1, in Zone 2 – in 70.4 [65.6; 75.8] %, which significantly ($p<0.01$) differed from Zone 1. In Zone 3, the percentage of casp8-positive neurons was 48.5 [42.9; 54.5] %. This also significantly differed from both indicators of Zone 2 samples and the control group (36.2 [28; 42.2] %).

Thus, during the acute phase of ischemic stroke, there is an increase in the proportion of neurons expressing apoptotic proteins in the cerebral cortex compared to the control group. A characteristic trend is a decrease in the proportion of p53-,

Concentrations of hormones of the stress-realizing system and soluble apoptosis-regulating factors of the Fas system in the peripheral blood of patients in the acute phase of IS and in the control group

Indicator	Patients after IS	Control group
sFas, pg/ml	124.3±51.0	92.3±26.6
sFasL, pg/ml	477.4±220.4*	208.3±67.1
sFasL/sFas, AU	3.8±0.5*	2.3±0.3
Cortisol, nmol/L	556.1±54.0*	247.0±45.7
ACTH, pg/ml	17.6±3.9*	7.9±1.9
Adrenaline, pg/ml	56.8±21.8	62.1±27.7
Noradrenaline, pg/ml	126.4±35.2	127.0±14.5

Note: AU – arbitrary units (dimensionless quantity); * – $p<0.01$ compared to the control value.



Proportion of p53 (a), casp3 (b), casp8 (c), Fas (d), FasL (e), FAIM2 (f)-positive neurons in cerebral cortex samples from patients with IS and the control group (K).
** – p-level of the difference between the data and the control (*p<0.05; **p<0.01);*
 # – p-level of the difference between the data and the previous zone (#p<0.05; ##p<0.01)

casp8-, and casp3-positive cells as the distance from the ischemic focus increases.

The proportion of neurons expressing membrane-bound forms of Fas receptors in the investigated Zone 1 was the highest (35.3 [30.4; 40] %) and significantly different (p<0.01) from the control group (7 [4.5; 10.3] %). In Zones 2 and 3, their numbers significantly decreased to 28.6 [24.1; 34.3] % and 10.4 [6.1; 15.2] %, respectively. Interestingly, only in Zone 3 did the increase in the proportion of Fas-positive neurons correspond to an increase in the proportion of casp8-positive neurons (r=0.174; p<0.05; figures *d–f*).

The expression of membrane-bound forms of Fas ligands was detected in 63.6 [58.3; 70] % of NSE-positive cells in Zone 1 and was significantly (p<0.01) lower than in the control samples (75.5 [66.7; 81.5] %). In contrast, Zones 2 and 3 showed a significant increase in the proportion of neuronal cells expressing FasL, reaching 78.1 [71.4; 85.7] % (p<0.05) and 89.1 [81.4; 93] % (p<0.01), respectively. The increase in the proportion of FasL-positive neurons with increasing distance from the ischemic focus may represent a mechanism of sanogenesis aimed at preventing interaction of other cellular elements with FasL by activating death receptors on them.

The proportion of FasL-positive non-neuronal cells in the control group was the lowest (11.8 [8.7; 16.3] %), which significantly differed from the values observed in the samples of the research group. Specifically, in Zone 3, this parameter was 15.4 [10; 20] %, and then the proportion of FasL-positive non-neuronal cells significantly increased (p<0.01) as the ischemic focus was approached. In Zone 2, it reached 19 [14.3; 23.8] %, and in Zone 1 it was 29.7 [26.4; 34.6] %.

The maximum representation of FasL-positive non-neuronal cells in Zone 1 can be explained by leukocytic infiltration.

In Zone 3, the following pattern was observed: a decrease in the proportion of Fas- and casp8-positive neurons correlated with an increase in the proportion of FasL-positive non-neuronal cells (correlation coefficient r=-0.160; p<0.05 and r=-0.211; p<0.01, respectively). No correlation was found between this parameter and the proportion of casp3-positive neurons. Therefore, in this zone, Fas-mediated apoptosis is not the main mechanism of neuronal death.

The proportion of FAIM2-positive neurons in Zone 2 was 73 [67.9; 78.9] % and did not significantly differ from the control group's values (72.4 [66.1; 76.8] %). In the other research zones, the differences from the controls were significant (p<0.01), with lower values. Specifically, in Zones 1 and 3, they were 47.1 [42.9; 52.9] % and 59 [52.9; 63.8] %, respectively. In the control group, there was a significant direct correlation between the proportion of neurons expressing FAIM2 and the proportion of p53-positive neurons (r=0.282; p<0.05), while in Zone 3, it was inverse (r=-0.177; p<0.05). For Zone 2, a negative correlation coefficient was obtained between the proportion of FAIM2-positive neurons and the proportion of neurons expressing casp3 (r=-0.183; p<0.05), and for Zone 1, it was with the membrane form of FasL (r=-0.148; p<0.05). This indicates that after cerebral ischemic injury, neurons in the brain cortex compete for the opportunity to implement adaptation mechanisms and programmed cell death. In control samples, there is parallel coexistence and synergy of these processes.

The prevalence and localization of neurons expressing apoptosis-executing proteins p53, casp8, casp3 in the brain cor-

tex did not correlate with the severity of neurological deficit, as expressed in NIHSS scores, for any of the investigated zones. This indicates a stereotypical response to ischemic injury regardless of its clinical severity.

No significant correlations were found between the proportions of p53-, casp8-, casp3-positive neurons.

The proportion of p53- and casp8-positive neurons was not influenced by the concentration of any of the investigated stress-effector systems or apoptosis-regulating factors in peripheral blood.

In Zones 2 and 3, significant correlation was found between the proportion of casp3-positive neurons and cortisol concentration in peripheral blood. With increasing cortisol concentration, the proportion of neurons expressing casp3 increased both in Zone 2 ($r=0.263$; $p<0.01$) and in Zone 3 ($r=0.383$; $p<0.01$). In Zone 2, significant negative correlations were observed with concentrations of sFas ($r=-0.177$; $p<0.05$) and sFasL ($r=-0.164$; $p<0.05$), while in Zone 3, significant positive correlations were observed with the ratio of sFasL and sFas concentrations ($r=0.240$; $p<0.01$).

The proportion of Fas-positive neurons significantly correlated with the concentration of the soluble form of this molecule and the ratio of sFasL to sFas concentrations in peripheral blood. This justifies the possibility of cross-prediction and extrapolation of data on the dynamics of soluble form concentrations and the presence of membrane forms of receptors and ligands of the Fas system in cortical neurons. For sFasL, the correlation coefficients were as follows: for Zone 1 – $r= 0.222$, for Zone 2 – $r=0.438$, for Zone 3 – $r=0.289$, $p<0.01$; for the ratio of sFasL to sFas concentrations -$r=0.231$, $r=0.266$, and $r=0.281$, respectively, $p<0.01$. However, for the membrane form of the Fas receptor system, as well as for FAIM2, the correlations with the aforementioned indicators were not significant.

Thus, during the acute period of ischemic stroke, the increased efficiency of soluble regulators of apoptosis execution by Fas system ligands corresponds to enhanced expression by neurons of death ligands of the Fas system and molecules FAIM2 associated with the Fas receptor, which are involved in inhibiting signaling pathways of the programmed cell death.

In correlation analysis with indicators of stress hormone concentrations in peripheral blood, the following significant correlations were found.

The proportion of Fas-positive neurons in samples of the brain cortex did not correlate with any of the investigated parameters.

In Zone 2, the maximum number of significant correlation links was observed. Thus, the proportion of FasL-positive neurons significantly correlated with the concentration of ACTH ($r=-0.362$; $p<0.01$), cortisol ($r=-0.212$; $p<0.01$), and noradrenaline ($r=0.410$; $p<0.01$). In the same zone, a negative correlation was found between cortisol concentration and the proportion of FAIM2-positive neurons ($r=-0.177$; $p<0.05$).

In Zone 1, among all the parameters considered, only the concentration of ACTH in peripheral blood and the proportion of FasL-positive neurons correlated ($r=-0.152$; $p<0.05$). In Zone 3, the proportion of FasL-positive neurons significantly correlated with the concentration of adrenaline ($r=0.219$; $p<0.01$), noradrenaline ($r=0.230$; $p<0.01$), and ACTH ($r=-0.264$; $p<0.01$) in peripheral blood.

The proportion of FasL-positive neurons in the brain cortex significantly correlated with the severity of neurological deficit, expressed in NIHSS scores, in all investigated zones (for Zones 1, 2, and 3: $r=0.191$, $r=0.427$, $r=0.294$, respectively; $p<0.01$). Additionally, the proportion of FAIM2-positive neurons in Zone 2 correlated with the severity of neurological deficit.

Discussion. Based on the obtained results, it can be concluded that in the acute phase of ischemic stroke, there are opposite patterns in the expression of FasL and Fas in the neurons of the cerebral cortex. These patterns consist in a decrease in the representation of Fas-positive neurons and an increase in the proportion of FasL-positive neurons as the distance from the ischemic focus increases. The lowest proportion of FAIM2-positive neurons in Zone 1 provides the highest probability of developing Fas-regulated apoptosis in these neurons. The maximum proportion of FAIM2-positive neurons in Zone 2 is associated with a higher presence of the Fas receptor in this area compared to Zone 3. This may also indicate that the processes of neuroplasticity, aimed at forming new connections between neurons, are most actively implemented here. Another important finding is the significant differences detected between the values of the investigated parameters in the control group and in Zone 3 located in the contralateral hemisphere. This suggests that changes due to local cerebral ischemia affect the opposite hemisphere.

The obtained findings lead to conclusions that in ischemic brain injury the effectiveness of the attack of Fas receptors by regulatory apoptosis ligands, including on the surface of neurons, increases (the ratio of concentrations of soluble forms of ligands and receptors increases in peripheral blood). In Zone 1, the low proportion of FAIM2-positive neurons may indicate a high probability of their response to such ligand-receptor interaction in the form of cell death, i.e., the range of possible adaptive solutions (possible variations in neuron functioning when the microenvironment changes) in this zone is narrowed. Thus, in Zone 1 compared to other investigated zones, the neuronal response to any stimulation is characterized by maximum rigidity. Therefore, this zone is not a promising therapeutic target, and attempts to preserve neuron viability in it are likely not advisable. The main therapeutic task is to optimize the delineation of the ischemic focus from viable, functionally active nervous tissue.

For Zone 2, an increase in the effectiveness of the attack of Fas receptors by regulatory apoptosis ligands is also characteristic, albeit to a lesser extent than for Zone 1. At the same time, the maximum proportion of FAIM2-positive neurons indicates a greater capacity of neurons than in other zones to respond to regulatory apoptosis stimuli by activating not the signaling cascades leading the cell to self-destruction, but those leading to adaptive changes of its structure and function (survival, synaptogenesis, sprouting, pruning). Thus, in Zone 2 compared to other investigated zones, the neuronal response to stimulation is characterized by a wide range of possible adaptive solutions. Zone 2, with its maximum sensitivity to the influence of regulatory apoptosis factors and the widest range of adaptive responses, has the greatest neuroplastic potential for restoring lost functional interneuronal connections. In this zone, a significant correlation was also noted between the proportion of casp3-positive neurons and the concentration of cortisol in peripheral blood. With increasing cortisol concentration, the proportion of neurons expressing casp3 increased. Considering the adaptive reserve of this zone and the negative

impact of cortisol level elevation on it, from a clinical perspective it seems reasonable to apply pharmacological and non-pharmacological therapies indirectly aimed at relatively reducing the level of this hormone in the blood: timely detection and treatment of depression, the use of cognitive-behavioral psychotherapy methods to develop active coping strategies characterized by the predominance of the sympathoadrenal axis in response to stress [18–20].

In Zone 3, due to the lowest representation of neurons expressing death receptors Fas and the highest representation of neurons expressing FasL, the likelihood of death receptor interaction on neurons with their ligands, followed by the implementation of intracellular signaling cascades leading to cell death, is reduced. This is confirmed by the fact that Zone 3 has the lowest proportion of p53-, casp3-, casp8-positive neurons. The proportion of FAIM2-positive neurons in this zone is significantly lower than in Zone 2, indicating a narrower range of possible adaptive solutions in Zone 3 compared to Zone 2.

Thus, the maximum modulating effect on the regulation of apoptosis mechanisms by stress-realizing system hormones is exerted in Zone 2. Additionally, considering the negative correlation coefficient of its concentration with the proportion of FAIM2-positive neurons, the increase in cortisol concentration leads to narrowing of the range of adaptive solutions and increases the likelihood of programmed cell death implementation (based on the positive correlation coefficients of cortisol concentration with the proportion of neurons expressing apoptotic proteins).

Conclusion. The concentration of cortisol in peripheral blood is a factor determining the regulation of apoptosis in neurons of the cerebral cortex during the acute phase of ischemic brain injury.

High levels of cortisol and ACTH concentration in peripheral blood are associated with an increase in the proportion of neurons implementing apoptosis mechanisms. The concentration of soluble Fas and the ratio of sFasL to sFas concentrations in peripheral blood significantly correlate with the proportion of Fas-positive neurons, thus justifying the possibility of cross-prediction and extrapolation of data on the dynamics of soluble form concentrations and the representation of membrane forms of Fas system receptors and ligands in neurons of the cerebral cortex.

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