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MODERN POSSIBILITIES OF DIAGNOSIS OF CELL'S MEMBRANE-RECEPTOR COMPLEX DYSFUNCTION IN THE ACUTE PERIOD OF ISCHEMIC STROKE

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During the development of the acute phase of ischemic stroke (IS), the cytoplasmic membranes of cells and receptor complex are primarily damaged. Structural changes in brain tissue are accompanied by violations of its electric physical characteristics, which are quite qualitatively possible to record using microwave dielectrometry.

The aim of the study *was to learn the features of changes in the dielectric properties of peripheral blood erythrocytes of patients with IS during in vitro exposure to adrenergic drugs depending on the severity of the disease to optimize diagnosis.*

Material and methods of research. *Measurements of complex dielectric constant (CDC) were performed by EHF dielectrometry at a fixed frequency of 39.5×109 Hz, which is in the region of γ-dispersion. The work was based on the materials of a comprehensive examination of 350 patients with the first in the life of IS on the 1st, 10th and 21st day of the disease.*

The severity of the condition and the degree of neurological deficit were determined using the stroke scale of the US National Institutes of Health (NIHSS) in the first hours of the disease, in the dynamics of treatment on the 10th and 21st day. There were 2 clinical groups: 1st (n=183) – patients in a state of moderate severity (mean score on the NIHSS scale 11.74±0.33); 2nd (n=167) – patients with severe IS (average score on the NIHSS scale 24.06±0.29).

Results. *In the control group, there was a significant decrease after exposure to adrenaline solution. The reaction to adrenaline in patients with IS was almost absent. It indicates a decreasing of erythrocytes β-adrenoceptors (β-ARs) sensitivity to stimulants due to their desensitisation. In patients with moderate severity, erythrocytes are more reactive than in patients with more severe IS.*

Conclusions. *The deviation of CDC of erythrocytes induced by adrenergic drugs in vitro is a manifestation of a specific response of cells to biologically active substances and depends on the functional state of the sympathoadrenal system*

Keywords: *complex, receptor, adrenal reactivity, membrane, ischemia, molecule*

1. Introduction

The cascade of pathological biochemical reactions in the centre of hypoxia during the development of the clinical picture of ischemic stroke (IS) such as oxidative stress, glutamate excitotoxicity, local inflammatory response and others, primarily leads to damage of external cytoplasmic membranes and receptors [1, 2].

The study of structural and functional changes in the parameters of brain tissue in conditions of acute cerebral ischemia, which correlate with violations of their electrophysical characteristics, opens broad prospects for the use of microwave dielectrometry in angioneurology [3, 4]. This is evidenced by the results of recent studies, which proved the presence of stable changes in the hydrated environment of biological molecules of damaged tissues, which can radically change cell metabolism [5, 6].

Because today the most informative biological tissue is blood with components, erythrocytes have been widely used as model systems for studying various mechanisms, including intracellular signalling from biologically active substances (BAS) of different natures [7]. Numerous studies show that the work of membrane-receptor complex (MRC) of blood cells reflects the functional state of the whole organism. One of the most important criteria for assessing is the adrenergic activity of cytoplasmic membranes, which also plays a leading role in the pathogenesis of IS [8, 9].

Calcium-dependent Na^+/K^+ channels, the mechanisms of regulation of which are directly related to the change in cell volume due to intracellular water transport, are involved in the processes of changes in blood properties induced by adrenergic drugs (ADs) [9]. In this regard, the use of EHF dielectrometry to study the hydrated state of cellular blood structures under the action of ADs on them is an auspicious and urgent task.

The aim of the research was a comprehensive study of features in changes of the dielectric properties of erythrocytes in peripheral blood of patients with IS during in vitro exposure to ADs depending on the severity of the disease for the diagnosis optimization.

2. Material and methods of research

Measurements of complex dielectric constant (CDC, Δε') were performed by EHF dielectrometry at a fixed frequency of 39.5×10^9 Hz (or 39.5 GHz, which corresponds to the EHF wavelength in free space λ=7.6 mm), which is in the region of γ-dispersion.

The technical feature of the waveguide method is that first the complex reflection coefficient is measured from the waveguide measuring structure – cuvettes with

a sample volume of 5–6 μl, after which the values of the reflection coefficient are converted into complex dielectric constant using specially designed programs which provide a measuring complex, which is a development in the O. Ya. Usikov Institute for Radiophysics and Electronics of the National Academy of Sciences of Ukraine.

Statistical processing of the results of experimental studies was performed using the program Statistics 6.0. Non-parametric statistical tests were used to assess the differences between groups (Wilcoxon rank-sum test), Kolmogorov-Smirnov and permutation test (two-sample permutation test).

The work was based on the materials of a comprehensive examination of 350 patients with the first in the life of IS on the 1st, 10th and 21st day of the disease in the period from 2014 to 2015 years. The criteria for selecting patients were: age from 46 to 79 years; the first in the life of IS with localisation in the pool of blood supply to the middle cerebral artery; hospitalisation of the patients up to 24 hours from the beginning of the disease; MRI results ruled out intracranial haemorrhage or other non-ischemic brain damage.

All human studies were conducted in accordance with the Declaration of Helsinki (1964, last review 64th WMA General Assembly, Brazil, 2013). The study protocol was approved by the Ethics Committee for Bioethics of Medical Institute of Sumy State University No. 33 from 22.04.2014.. Patients or their relatives gave informational consent to participate in the study.

Males accounted for 185 patients (52.8 %), females – 165 (47.2 %). The mean age of the examined patients was 65.2±0.7 years (range from 46 to 79 years). As a comparison group, an additional 30 relatively healthy individuals (17 men and 13 women, mean age – 60.4±0.8 years), who are homogeneous in sex and age with a group of patients with IS were examined.

The severity of the condition and the degree of neurological deficit were determined using the stroke scale of the US National Institutes of Health (NIHSS) with a score of 15 indicators, expressed in points in the first hours of the disease, in the dynamics of treatment on the 10th and 21st day.

Based on this, all patients were divided into 2 clinical groups: 1st (n=183) – patients in a state of moderate severity (mean score on the NIHSS scale 11.74±0.33); 2nd (n=167) – patients with severe IS (mean score on the NIHSS scale 24.06±0.29).

3. Results of the research

Measurements of CDC were performed under the influence of adrenaline on erythrocytes of relatively healthy individuals (10^{-7} M) to identify their response to stress factor with a representation of the relative values of the real part of dielectric constant, which is the difference between the measured CDC before and after exposure to ADs (Table 1).

It was found that the relative values of Δε' were differentiated by groups. In the control group, there was a significant decrease after exposure to adrenaline solution. In patients with IS, the reaction to adrenaline was almost absent, which indicates a decrease in the sensitivity of β-adrenoceptors (β-ARc) of erythrocytes to stimulants due to their desensitisation. However, the value of Δε' in the group of patients with moderate severity shows that their erythrocytes are more reactive than in the group of patients with a more severe variant of IS.

Table 1

Dynamics of values of Δε' erythrocyte suspensions after exposure to adrenaline in patients with IS depending from the severity of the disease

Group of patients	The term of the study	The value of Δε'±s
1 group	1st day	0.21±0.08
	10th day	0.33±0.04
	21st day	0.35±0.1
2 group	1st day	–0.48±0.18
	10th day	–0.33±0.09
	21st day	–0.31±0.04
Control group	–1.21±0.15	

Note: all indicators are reliable for control ($p<0.05$)

A similar effect was also observed when comparing the dynamics of changes in the values of Δε 'erythrocyte suspensions under the influence of β-adrenoblocker (β-AB) propranolol depending on the severity of IS. Effects in the control group in 100 % of cases had a negative sign, the magnitude of the impact in 44 % of cases exceeded 10 % relative to samples with an equal volume addition of 0.9 % saline. In the group of patients with severe IS, all changes were within error, and in the group of patients with moderate severity of the disease there was a reaction to β-AB; however, the sign of the effect was opposite to the control indicator, as in the case of adrenaline.

As for the combined action of adrenaline and propranolol, when the adrenaline was added after previous incubation of cells with β-AB, the reactions were observed only in 46.3 % of cases in the group of patients with moderate IS (Table 2). No reactions in the control group were expected due to previous β-ARs blockade.

In all samples of patients with moderate severity, there was a tendency to increase the absolute values of CDC on the background of the prescribed treatment, which indicates changes in the levels of hydration of erythrocytes. All this suggests an increase in the amount of free water in the samples and a decrease in the amount of bound. This assumption can be made based on the fact that in the dynamics of disease decreases the thickness of the membrane layer up to 20 %.

This affects the rate of metabolic processes in the system «cell-intercellular environment», thereby changing the mode of functioning of both the cell and its MRC.

Correlation analysis of the relationship between values of CDC and the total clinical score on the NIHSS scale showed some features (Fig. 1).

Table 2

Dynamics of values of Δε' erythrocyte suspensions under the action of adrenaline after incubation with propranolol in patients with IS depending from the severity of the disease

Group of patients	The term of the study	The value of Δε'±s
1 group	1st day	0.28±0.03
	10th day	0.29±0.06
	21st day	0.24±0.05
2 group	1st day	0.88±0.18
	10th day	0.92±0.13
	21st day	0.94±0.07
Control group	0.18±0.02	

Note: all indicators are reliable for control ($p<0.05$)

An inverse correlation was found between the values of Δε' and the level of the neurological deficit on the NIHSS scale in patients with IS at the onset of the disease ($r=-0.83$; $p<0.05$). In the dynamics of observation between these indicators also remained a correlation, which did not lose its strength and direction ($r=-0.77$; $p<0.05$).

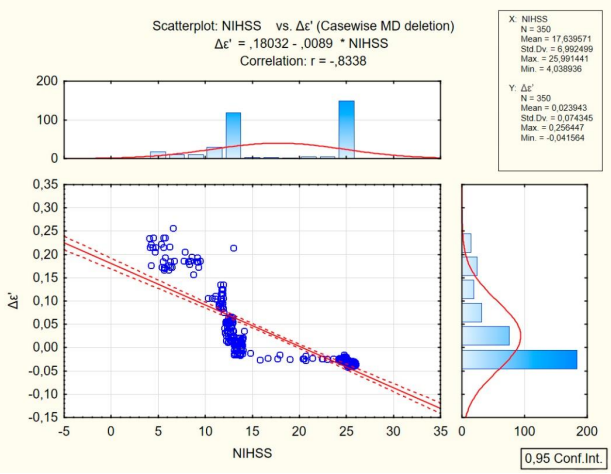

Fig. 1. Correlation between the values of CDC and the degree of the neurological deficit on the NIHSS scale in patients with IS during hospitalisation

4. Discussion

If we talk about the mechanisms of membranes adrenergic reactivity (MAR) disruption at the reception level, then, in addition to the molecular processes associated with polymorphism of ARs, there are also various phenomena of desensitisation such as phosphorylation, sequestration and down-regulation [10].

Endogenous hypercatecholaminemia, which always occurs in the acute period of IS, can cause desensitisation of β-ARs with a decrease in total MAR. Probably, the elimination of this mechanism is associated with some increase in the number of β-ARs at the end of the acute period of the disease [11].

The study showed that during IS, there is a significant increase in the liquid properties of the lipid matrix of membranes. It provides lateral mobility of receptor molecules in the membrane plane, on which the convergence of β-APs receptor subunits depends, and also determines with which type of G-protein the receptor will bind [12].

Considering the membrane-receptor mechanisms of regulation of pathological reactions during IS, we cannot dwell on participating in the transmission of a transmembrane signal of ion channels and, above all, Ca^{2+} channels, because in most cases this signalling is provided by phosphoinositide mechanism and increasing intracellular calcium concentration [13].

Given the above, we can conclude that the violation of MRC in IS is not only the central pathogenetic link of the disease, which determines the adequacy of functional activity of the cell concerning external information signals but also have a primary, universal nature, suggesting acute cerebral ischemia as primarily membrane-receptor pathology

The nature of pathological changes in the area of IS in combination with the above factors provides a significant impact on the rate and degree of damage to brain tissue. It can also be a prognostic indicator of disease outcome.

Study limitations. The study included a small cohort of patients with IS in combination with pulmonary insufficiency, followed by studies of the largest cohort of patients. Adults dominated in the study.

Prospects for further research. In the future it is planned to study the effectiveness of neurotrophic factors on the adrenergic activity of erythrocytes in patients with ischemic stroke.

5. Conclusions

For the first time, an informative integrated approach for the evaluation of β-ARM of peripheral blood erythrocytes in patients with IS was developed, which is based on the analysis of changes in their dielectric characteristics under the action of ADs. Deviations of CDC of the erythrocytes which were induced by adrenergic substances in vitro are the display of specific reaction of cells to biologically active substances and depend on a functional condition of the sympathoadrenal system.

On the onset of the disease, it is recommended to measure the values of CDC erythrocyte suspension under the influence of adrenaline, which on the 1st day is from – 0.51±0.14 to 0.21±0.08. Levels from 0.44±0.12 to 0.35±0.1 should be considered harmful for the prognosis.

During hospitalisation, it is recommended to determine the values of CDC of erythrocyte suspension under the influence of propranolol, which on the 1st day can be from – 0.39±0.11 to 0.91±0.15. Levels from 1.02±±0.09 to 1.31±0.14 should also be considered harmful for disease outcome.

Conflict of interest

The authors declare that they have no conflicts of interest.

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