

**THE NATURE OF BIOCHEMICAL SHIFTS IN RED BLOOD CELLS UNDER THE
INFLUENCE OF RADIO FREQUENCY ELECTROMAGNETIC RADIATION RANGE
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SUMMARY

An experimental study on 72 white rats was carried out, which were divided into 4 groups: 1, 2, and 3 experimental groups of 20 animals in each with the power of radio frequency electromagnetic radiation (RFEMR) 50, 500 and mW/cm^2 , respectively. Group 4 was a control group of 12 animals, without affecting RFEMR. The dynamics of the processes of free radical oxidation in the structures was studied that are most sensitive to the effects of RFEMR in erythrocytes. Based on the results of the study found that exposure to RFEMR within one month accompanied by more pronounced changes of the studied parameters in the red blood cells, in particular, destruction of membrane structures due to accumulation inside the cell peroxidation products compared with three months exposure.

KEYWORDS: Electromagnetic radiation, experiment, lipid peroxidation, antioxidant protection.

The scientific works of domestic and foreign experts provide a lot of information about the nature of changes in the human and animal body under the influence of electromagnetic radiation in the radio frequency range (RFEMR). Professionals operating radio systems are exposed to multi-frequency modulated electromagnetic radiation. However, targeted comprehensive studies of the biological effects of RFEMR, as well as the effect of this radiation on the functional state of red blood cells and a number of biochemical processes in them, were not carried out.

Given the insufficiency, and often the inconsistency, of the available information, the aim of this study was to study the effect of various capacities and exposures of EMIR on certain biochemical processes in the body of experimental animals in a comparative aspect.

To achieve this goal, we studied the dynamics of a number of biochemical processes, including free radical oxidation in the structures that are most sensitive to the effects of RFEMR - in red blood cells.

Materials and research methods. To achieve this goal, we studied the dynamics of lipid peroxidation (lipid peroxidation) and anti-radical defense factors in red blood cells under the influence of RFEMR.

An experimental study was conducted on 72 white rats, which were divided into 4 groups: 1, 2, and 3 experimental groups of 20 animals each with an RFEMR

power of 50, 500, and $1000 \mu W / cm^2$ respectively. 4 group - control (12 animals), without exposure to RFEMR.

Our results reflect the peculiarities of the lipolytic enzyme Ar phospholipase Ar, lipid peroxidation and antioxidant defense (AOD) in rats during acute (1 month) and chronic (3 months) exposure to RFEMR.

LPO processes were studied by the indicator of its final product - malondialdehyde (MDA), and AOD factors - by the level of catalase and superoxide dismutase (SOD).

For the study used venous blood taken in a ratio of 9: 1 with a 3.8% solution of sodium citrate. To obtain an erythrocyte suspension, erythrocytes were washed three times with physiological saline followed by centrifugation for 15 minutes. at 2000 rpm Erythrocyte hemolysis was carried out by adding an equal amount of distilled water to them. 0.2 ml of a 2M sodium chloride solution was added to 0.4 ml of hemolysate and stirred for 10 minutes. at $37^{\circ}C$ for complete extraction of phospholipase. An enzyme solution was prepared by mixing different volumes of the obtained extract and three HCl buffers.

The activity of phospholipase was evaluated by the degree of clarification of the lecithin emulsion (10% solution of lecithin in a solvent consisting of 95% ether and methanol). The optical density of the control sample versus the experimental one at 500 ml and the specific

activity of phospholipase A in blood erythrocytes were calculated in the ratio of 1 mg of hemoglobin.

To determine the content of malondialdehyde (MDA) used the method of L.I. Andreeva et al. (1988). During the study, the optical density of the prototype was measured at a wavelength of 535 nm. The results of the study were calculated using the molar extinction coefficient MDA and expressed in $\mu\text{mol/L}$.

The study of catalase activity was carried out according to the method of M.A. Korolyuk et al. (1989). The color intensity during the study was measured on a spectrophotometer at a wavelength of 410 nm. Catalase activity in blood erythrocytes was expressed in $\text{mol} / \text{min} / \text{mg}$ hemoglobin.

Studies of the activity of superoxide dismutase (SOD) in blood red blood cells were carried out according to the method of E.E. Dubinina et al. (1983). The optical density of the test sample was measured at a wavelength of 540 nm. SOD activity was expressed in arbitrary units relative to mg of hemoglobin.

Results and discussion. In acute erythrocytes of rats (acute duration of RFEMR exposure of 1 month), a significant increase in the intensity of LPO processes under the influence of RFEMR was revealed.

Based on the data presented, it was found that as the dose of RFEMR increases, there is a dynamic increase in the level of MDA. So, with irradiation of $50 \mu\text{W} / \text{cm}^2$, an increase in the MDA level to $0.07 \pm 0.01 \mu\text{mol} / \text{L}$ was noted, compared with the performance in intact rats - $0.05 \pm 0.002 \mu\text{mol} / \text{L}$ ($P > 0.05$). As the RFEMR power increases from $500 \mu\text{W} / \text{cm}^2$ to $1000 \mu\text{W} / \text{cm}^2$, an even greater increase in the MDA level in erythrocytes is noted (0.10 ± 0.004 and $0.12 \pm 0.011 \mu\text{mol} / \text{L}$, respectively). These indices not only significantly exceeded those in intact rats ($P < 0.001$), but also indices in animals from the 1st group ($P < 0.05$). As established, the most significant changes in the MDA are noted at high and RFEMR of very high power.

Even more significant changes were observed in rats in terms of AOD. So, the level of SOD exceeded that of intact rats by almost 2 times when exposed to RFEMR of $50 \mu\text{W} / \text{cm}^2$, which may indicate the inclusion of adaptation processes on the activation of LP. However, under the influence of RFEMR of 500 and $1000 \mu\text{W} / \text{cm}^2$, the activity of SOD decreased, which may indicate an overwhelming effect on the AOD of the indicated doses of RFEMR.

When analyzing a number of other indicators of antioxidant protection (catalase, glutathione peroxidase) in the framework of an acute experiment, we revealed a similar orientation of the effects of RFEMR, but the differences in the compared parameters were less significant than the SOD indicators compared to intact

animals and depending on the radiation dose. In general, during the entire acute experiment, an increase in LP was observed, and at high power EMIRCH (500 and $1000 \mu\text{W}/\text{cm}^2$), a decrease in AOD activity was noted.

There is evidence that RFEMR has a significant effect on the activation of a number of biochemical parameters in humans and animals.^[1,2,7,10,11] It was to be expected that a similar effect would be exerted by this type of radiation on the activity of phospholipase-Ar. The aforesaid is confirmed by the results obtained by us when studying the dynamics of phospholipase-Ar activity in red blood cells of experimental animals.

So when exposed to RFEMR of $50 \mu\text{W} / \text{cm}^2$, the studied indicator exceeded the initial level by almost 1.5 times. However, in the future, when the dose of EMIR is increased to $500 \mu\text{W} / \text{cm}^2$, the level of phospholipase-Ar exceeded the initial values to a lesser extent - 1.3 times. When exposed to an RFEMR of $1000 \mu\text{W} / \text{cm}^2$, the level of phospholipase-Ar exceeded that of intact animals by almost 2 times. The effects obtained suggest that RFEMR has a significant effect on the activity of phospholipase-Ar in the blood erythrocytes of experimental animals. At the same time, the maximum values of this indicator were observed under the influence of an EMRCH of $1000 \mu\text{W} / \text{cm}^2$.

Enhanced lipid peroxidation of erythrocyte membranes is accompanied by a change in membrane permeability, which leads to the accumulation of calcium ions inside the cell, which activates the lipolytic enzyme phospholipase-Ar. As can be seen from the results of the study, under the influence of RFEMR in acute experience, a significant increase in the activity of phospholipase-Ar is observed, which contributes to the accumulation of phospholipid lysoforms, toxic metabolites of the breakdown of membrane phospholipids.^[12]

Next, we analyzed similar biochemical parameters at various doses of RFEMR in a chronic experiment (exposure duration 3 months). As can be seen from the presented results of the study, the level of MDA in the blood erythrocytes in experimental rats increases significantly when exposed to RFEMR equal to $50 \mu\text{W} / \text{cm}^2$ and was equal to $0.14 \pm 0.003 \mu\text{mol} / \text{L}$ hemoglobin, which is 2.8 times higher than in intact rats ($P < 0.05$). An increase in RFEMR to $500 \mu\text{W} / \text{cm}^2$ is accompanied by a slight decrease in this indicator compared to the previous group of animals, but at the same time it exceeded the initial MDA level by 2.6 times ($P < 0.01$). The highest values of MDA in the blood erythrocytes of experimental animals were observed when exposed to RFEMR equal to $1000 \mu\text{W} / \text{cm}^2$, which averaged $0.15 \pm 0.01 \mu\text{mol} / \text{L}$ and exceeded not only the MDA value in intact rats by 3 times ($p < 0.001$), but also the values of this indicator in rats from groups 1 and 2 (1.1 and 1.15 times, respectively), although not so significant ($P > 0.05$ and $P < 0.05$, respectively).

When studying the dynamics of phospholipase-Ar activity in the blood erythrocytes of experimental animals in a chronic experiment under the influence of RFEMR equal to $50 \mu\text{W} / \text{cm}^2$, the studied parameter exceeded the initial level by 6%, at $500 \mu\text{W} / \text{cm}^2$ - by 31% and at $1000 \mu\text{W} / \text{cm}^2$ - by 57 %, respectively ($P > 0.05$ - < 0.001). Consequently, under the influence of RFEMR in a chronic experiment, activation of phospholipase-Ar is observed, which leads to significant destructive processes that begin with membrane structures.

The next stage of the study was an analysis of the activity of SOD under the influence of RFEMR. It was found that chronic experience is accompanied by a decrease in SOD activity when the experimental animals are exposed to $50 \mu\text{W} / \text{cm}^2$ of electromagnetic radiation by 32%, when $500 \mu\text{W} / \text{cm}^2$ by 46% and when $1000 \mu\text{W} / \text{cm}^2$ of electromagnetic radiation is 74% ($p < 0, 05$).

Analysis of the results of the study showed a tendency to increase the activity of catalase in experimental animals. So when exposed to RFEMR of $50 \mu\text{W} / \text{cm}^2$, it was equal to $1.04 \pm 0.05 \text{ mmol} / \text{mgNv}$, which was 35% higher than the initial level. Under the influence of RFEMR of $500 \mu\text{W} / \text{cm}^2$, the excess of the initial level was 31%, while, when exposed to RFEMR of $1000 \mu\text{W} / \text{cm}^2$, this indicator decreased by 24% compared with the control group.

In our studies, under the influence of RFEMR in experimental animals, we observed a dynamics of a decrease in glutathione reductase ($p < 0.001$), against the background of an increase in the activity of glutathione peroxidase, especially when exposed to RFEMR $50 \mu\text{W} / \text{cm}^2$ ($p < 0.05$). In this situation, glutathione peroxidase reacting with aldehydes formed during lipid peroxidation protects SH-groups of membrane proteins.

In our studies, superoxide dismutase provides protection against oxidation, intracellular reduced glutathione, which in the reduced state is an effective trap for free radicals. At the same time, a decrease in the activity of the glutathione antioxidant defense unit when exposed to RFEMR equal to $500\text{-}1000 \mu\text{W} / \text{cm}^2$ indicates the depletion of the most effective mechanism of protection against oxidative damage and thereby contributes to the aggravation of destructive oxidative processes.

A similar dynamic was noted with respect to the activity of SOD, which ensures the removal of a destructive superoxide anion radical from the erythrocyte with the formation of hydrogen peroxide. In a chronic experiment, experimental animals showed an increase in SOD activity by a factor of 2 compared with the control group.

Naturally, the accumulation of hydrogen peroxide in the cell under the influence of RFEMR and with the participation of SOD leads to a compensatory increase in

the activity of catalase in the blood erythrocyte by an average of 29% ($p < 0.001$) and glutathione peroxidase by 1.9 times ($p < 0.05$), which resists destructive processes.

When discussing the results of the studies, it should be pointed out that, as you know, chemical processes occur inside red blood cells that are involved in the production of energy for the needs of the cell. This energy creates an electromagnetic field around each red blood cell, and when merged from all cells, together they form an electromagnetic field around a person of certain frequencies (40-70 GHz). And if a person is also exposed to external electromagnetic radiation at these frequencies, the power of which is above a certain level, then the person's own electromagnetic field develops, as a result of which violations of chemical processes in red blood cells occur. In this situation, the biological effect of RFEMR depends on the duration, nature and mode of exposure.

Erythrocytes, in close contact with all tissues and entering into morphofunctional relationships with them, reflect their physiological and pathological changes in their own qualitative and quantitative restructuring. The multifunctional role of red blood cells in the mechanisms of adaptation and compensation under the influence of RFEMR explains the high information content of the results of studying functional changes in these cells.^[1,2,7,10,11]

Our results reflect the characteristics of the lipolytic enzyme, lipid peroxidation (lipid peroxidation) and antioxidant defense (AOD) in experimental rats under acute (1 month) and chronic (3 months) exposure to RFEMR.

It is known that under the action of phospholipase-Ar hydrolysis of phospholipids occurs with the formation of lysophospholipids - toxic metabolites of the decay of phospholipids. The degree of these effects is largely determined by the activity of phospholipase-Ar.^[2]

The activation of lipid peroxidation in blood erythrocytes is probably associated with inhibition of the activity of antioxidant enzymes, which subsequently affects the value of the electric breakdown potential of the erythrocyte membrane by reducing its stability and integrity.^[5,6,8,9]

It is known that the main oxygen-transporting protein, erythrocyte hemoglobin, is located in a medium with a higher oxygen concentration than most other cells.^[1,3,4,12] Red blood oxygen consumption is limited by daily auto-oxidation of hemoglobin to methemoglobin, which is associated with the formation of a destructive oxygen radical — the superoxide anion radical and toxic oxygen intermediates such as H_2O_2 , $\text{OH}\cdot$, HOCl , which cause oxidative stress with damage to cell membranes. Therefore, the activation of lipid peroxidation leads to "destruction" of the erythrocyte membrane, which affects

its physical state.^[5,6,9] The direct result of the accumulation of lipid peroxidation products is an increase in the permeability of the erythrocyte membrane, which may be due to a change in the charge on the interface / near the membrane medium, a change in the conformation of the membrane lipoprotein complexes, and the appearance of hydrophilic sites in the hydrophobic layer of the membrane. A decrease in the stability of the lipid bilayer and changes in the electrical properties of the membrane can lead to the loss of its barrier functions.^[5,8]

During peroxidation of the lipid layer of erythrocyte membranes, the functional activity of the calcium pump is disrupted, leading to the accumulation of calcium inside the cell, activation of phospholipase-A₂ located in the inner membrane.^[7] Under the influence of RFEMR in an acute experiment, phospholipase-A₂ activation is observed, which increases not only with increasing radiation power, but also with increasing exposure duration in a chronic experiment. It is known that activation of phospholipase-A₂ leads to the hydrolysis of the main phospholipids, choline and ethanolamine phosphatides, which carry a structural load in the architectonics of the erythrocyte membrane.^[8,10] This, in turn, can lead to serious impairment of red blood cell function.

In the erythrocyte cytoplasm there are also trace elements and antioxidant enzymes Cu, Zn, SOD, which ensure the removal of redox from the erythrocytes by destructive superoxide anion with the formation of hydrogen peroxide, which is destroyed by catalase and glutathione peroxidase.^[5,13]

According to modern concepts, the thesis about the most important protective role of SOD is not so obvious, since the toxicity of its substrate of superoxide anion radical is not very high. Hydrogen peroxide, which has a cytotoxic effect and the mechanisms of which are very diverse, is formed as a result of the superoxide dismutase reaction, is much more dangerous.^[5,12] In particular, under the action of H₂O₂, inactivation of catalase and glutathione peroxidase can be observed.^[13]

Glutathione is known to be present in the body in oxidized and reduced form. Glutathione is an activated oxygen radical inhibitor and membrane stabilizer.^[13] The resulting hydrogen peroxide, which is the strongest oxidizing agent, is partially neutralized non-enzymatically with the direct participation of the antioxidant - reduced glutathione. Most of the H₂O₂ is cleaved in reactions catalyzed by glutathione peroxidase. This is due to the fact that SH-containing compounds undergo oxidation in the first place, which prevents other functional groups from oxidizing. When oxidized glutathione is reduced, glutathione reductase forms H₂O₂, which is destroyed by catalase and glutathione peroxidase.

In general, it was found that in acute experience, i.e. for 1 month, more pronounced changes in the studied parameters in blood erythrocytes are observed, in particular, destruction of cell membranes due to the accumulation of lipid peroxidation products inside the cell, compared with the chronic exposure to RFEMR, in which adaptive processes seem to take effect. adaptive in nature, reducing the degree and nature of the severity of the destructive effects of RFEMR. However, in our opinion, with a longer chronic effect of RFEMR on these biochemical tests, one can expect the development of a breakdown of adaptation and activation of cell destruction processes. Similar results were obtained in the study of the effect of electromagnetic radiation on the brain cells of experimental animals and lipid peroxidation in blood serum.^[10]

Thus, under the influence of RFEMR, significant changes in biochemical metabolism occur, the degree of which is limited by the radiation power, but not by its exposure, as well as by the nature of adaptive-adaptive processes in the body of experimental animals and can determine the destructive-degenerative processes in it.

Enhanced lipid peroxidation of erythrocyte membranes is accompanied by a change in their permeability, which leads to the accumulation of calcium ions inside the cell, which activates the lipolytic enzyme phospholipase - A₂ and can increase the degree of destruction of cell membranes.

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