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Response of Erythrocyte Membranes to Total Body Exposure by 1800 MHz Frequency Microwave Radiation

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Abstract. Red blood cells were used to detect possible membrane perturbations as effects of rats total body exposure to 1800 MHz radiofrequency electromagnetic radiation (RF-EMR) using 2 different schemes of irradiation: 2-hour single and fractional exposure during 4 consecutive days for 0.5 hour daily. The following characteristic indices of erythrocyte membrane functional indices were analyzed: total permeability for K^+ ions, activity of specific Ca^{2+}-activated K^+-channels, as well as membrane potential and lipid peroxidation intensity. Our research revealed a biological response of erythrocyte membrane to 1800 MHz frequency RF-EMR at both schemes of irradiation manifested as alterations in functional properties after animal whole body exposure. Moreover, long-lasting character of these effects was revealed.

Keywords: 1800 MHz, electromagnetic radiation, rats, erythrocytes, membranes

Introduction

The widespread use of the Global System for Mobile Communications (GSM) made mobile phones indispensable as communication tools. Since the use of mobile phones and related technologies will continue to increase for the near future, any consequent biological effects of radio-frequency electromagnetic radiation (RF EMR) transmitted by mobile phones are still matters of public and scientific discussion. The concerns relate to the emissions of EMR from the mobile phones and base stations that receive and transmit the radio-frequency signals at frequencies in the microwave range of 900-1800 MHz.

Numerous experimental and epidemiological data testify that the ever-growing exposure of living organisms to RF-EMR promotes the occurrence of different disturbances in functional activity of cells and organs, which can stimulate development of several sicknesses in humans [1, 2].

At present, plasma membrane in cells is identified as the main target of RF EMR biological effects [3-5]. The goal of study was to investigate membrane effects of white rats at whole body exposure by low intensity radiation simulating GSM signal with 1800 MHz frequency.

Materials and Methods

1. Experimental design. Albino Wistar rats weighing 180-200 g were acclimated to the vivarium for at least 5 days before experimental manipulations. Two schemes of rats whole body exposure were considered in order to evaluate biological effects of low intensity 1800 MHz frequency EMR: 1) single acute exposure during 2 hours: n=24; 2) fractional exposure to RF EMR during 4 consecutive days for 0.5 hour daily: n=24. Animals kept at standard vivarium conditions served as Norm: n=10.

In order to perform irradiation, each time the groups of 12 animals were placed in a well-ventilated plastic box (25x22x15 cm^3) and exposed to whole body RF EMR with power density calculated as 8 $\mu W/cm^2$. Generating unit G4-81 served as a source of microwaves with 1800 MHz frequency. Fractal type Minkowski compact antenna (7.5x7.5 cm^2) served as

an irradiator with the resonance frequency equal to 1800 MHz, corresponding to the frequency generally used in cell phone communication.

On days 1, 5, 10, and 20 after the last exposure rats were sacrificed (6 rats per observation day) and blood samples were drawn for analyses. Data obtained were compared with those of Norm. Fresh heparinized blood from rats was centrifuged at 3000 rpm for 10 min. After removing plasma and buffy coat, pellets were washed three times with 0.9% NaCl. Resulting packed erythrocytes were re-suspended in saline to determine the indices characterizing membrane functional state.

2. K^+ permeability across the erythrocytes membranes, P_{K+} coefficient. Coefficient of K^+ permeability across the erythrocytes membranes P_{K+} was determined by ionometer "Multitest" and K^+-selective electrode ("SEMIKO", Novosibirsk) based on the results of K ions concentration increase in medium containing 0.1 ml of packed erythrocytes and 2.9 ml of 0.9% NaCl isotonic solution 1 hour after incubation at 37ºC. The value of P_{K+} is expressed in units $x10^{-9}$ cm/sec.

3. Activity of the Ca^{2+}-activated K^+-channels of erythrocytes, $P_{Ca2+-K+}$. Functional state of the Ca^{2+}-activated K^+-channels of erythrocytes $P_{Ca2+-K+}$ was evaluated using the method described by I.M. Glynn and A.E. Warner [6]. Briefly, packed erythrocytes in the volume of 0.1 ml are added to the solution containing 2.6 ml of saline and 30 microliters of $CaCl_2$. Using the K^+-selective electrode, the K^+ concentration in the medium is measured under continuous stirring. Then 0.3 ml of 0.1% Propranolol /ISIS PHARMA, Germany/ is added to the medium in order to determine the activity of Ca^{2+}-activated K^+-channels of erythrocytes, since in the presence of calcium in suspension medium Propranolol specifically activates those channels inducing an essential K^+ reflux from cells precisely through these channels. After Propranolol addition, K^+ concentration is measured continuously during 5 minutes. Judgment on the activity of Ca^{2+}-dependent K^+-channels is done by determination of erythrocytes permeability for K^+ based on calculation of the K^+ outflow rate into the incubation medium in the units $x10^{-9}$ cm/sec.

4. Membrane potential of erythrocytes, E_m. Erythrocyte membrane potential E_m was determined using the method of R.J. Macey et al. [7] that over a wide range does not depend on the hematocrit value and is based on the electrometric determination of extracellular/intracellular distribution of hydrogen ions. The pH of the medium containing 0.1 ml packed erythrocytes re-suspended in 2.9 ml of saline is measured twice: (a) immediately after the preparation of the sample (pH_1); (b) after hemolysis by adding of 0.2 ml of 0.2% saponin (pH_2). The obtained pH values are used in the Nernst formula ($E_m = -0.058 \lg [H]_1/[H]_2$) appropriately modified to $E_m = -58 (pH_1 - pH_2)$, where E_m is the membrane potential value in mV.

5. Lipid peroxidation intensity in erythrocytes, LPO_{er}. Analysis of the intensity of lipid peroxidation processes in erythrocytes (LPO_{er}) is based on determination of thiobarbituric acid reactive substances including malone dialdehyde (MDA) [8]. Briefly, the mixture containing 2 ml of a 5% erythrocyte suspension, 0.16 ml of 0.2% sodium aside solution, and 2 ml 0.068% H_2O_2 in buffered saline (pH 7.4), is incubated at 37ºC for 1 h. After incubation, 2 ml of 28% trichloroacetic acid is added. Then the mixture is filtered. Upon addition of 1 ml 1% of TBA prepared with 0.05 M NaOH to 4 ml of filtrate, the mixture is heated for 15 min. The concentration of MDA is determined spectrophotometrically at 532 nm. MDA content is calculated by molar extinction coefficient: $\varepsilon = 1.56 \times 10\text{-}5$ $moles^{-1}cm^{-1}$. The results are expressed as nanomoles MDA per 1 ml of erythrocyte suspension.

Ethical Guidelines. Studies in animals were approved by the Ethical Committee for Animal Care and Use in Biomedical Research at the Scientific Centre of Radiation Medicine

and Burns (Yerevan, Armenia) and performed according to the Guidelines of the International Association for the Study of Pain as published in the journal "Pain" [9]

Results and Discussion

Due to the unique bioelectrochemical properties, cell membranes are considered as the most likely acceptors of microwave radiation. Erythrocytes being a nucleated represent a reliable and easily obtainable model for measuring membrane properties without the interference of intracellular membranes. We used red blood cells (RBCs) to detect possible membrane perturbations as effects of rats whole body exposure to 1800 MHz frequency RF EMR analyzing such characteristic indices of erythrocyte membrane functional indices as the total permeability for K^+ ions, activity of specific Ca^{2+}-activated K^+-channels, as well as the membrane potential and LPO intensity.

The results of our study demonstrated that the organism exposure to RF EMR with 1800 MHz frequency resulted in the enhancement of K^+ leakage from erythrocytes, however, the onset and duration of registered effects depended on the mode of exposure (Table 1).

Table 1. Erythrocytes membrane K^+ permeability (**P_{K+}**) on Days 1, 5, 10 and 20 after the rats exposure to 1800 MHz frequency RF-EMR (Norm: 3.00±0.28 x 10^{-9} cm/sec)

Mode of exposure	Days after the exposure			
	1	5	10	20
Single	2.86±1.27	2.88±0.17	3.10±0.46	4.10±0.35*
Fractional	4.65±0.18*	2.95±0.14	5.6±0.39*	4.27±0.38*

Note: *- $p<0.05$

Thus, in the group of animals exposed to the single 2-hour 1800-MHz radiation, statistically significant increase of P_{K+} was registered only at day 20 after the treatment. In case of the fractional exposure, an essential rise of total K^+ outflow rate was recorded in all observation periods, besides Day 5 (Table 1), i.e. fractional mode of the exposure induced long lasting effects, which appeared just after the termination of the organism exposure. Changes in K^+ permeability might evidence certain alterations in structural composition of erythrocytes membranes that might be stimulated by 1800-MHz frequency RF EMR.

It is known that erythrocyte membrane contains one type of ion channels, namely Ca^{2+}-dependent K^+-channels. On the inner side of the cell membrane there are specific $Ca2^+$-dependent receptors sensitive to intracellular Ca^{2+} concentration increase, activation of which leads to K^+ outflow from RBCs through the Ca^{2+}-dependent K^+-channels [10]. Ca^{2+} is a universal signaling molecule involved in regulating cell cycle and fate, metabolism and structural integrity, motility and volume. Like other cells, RBCs rely on Ca^{2+} dependent signaling during differentiation from precursor cells. Intracellular Ca^{2+} levels in the circulating human RBCs take part not only in controlling biophysical properties such as membrane composition, volume and rheological properties, but also physiological parameters such as metabolic activity, redox state and cell clearance. Extremely low basal permeability of the human RBC membrane to Ca^{2+} and a powerful Ca^{2+} pump maintains intracellular free Ca^{2+} levels between 30 and 60 nM, whereas blood plasma Ca^{2+} is approximately 1.8 mM. Thus, activation of Ca^{2+} uptake has an impressive impact on multiple processes in the cells rendering Ca^{2+} a master regulator in erythrocytes. Malfunction of Ca^{2+} transporters leads to

excessive accumulation of Ca^{2+} within the cells. This is associated with a number of pathological states including different forms of hereditary anemia [11 and cited references].

According to data obtained in this study, both schemes of rats exposure to 1800-MHz frequency RF EMR produced essential changes in the activity Ca^{2+}-dependent K^+-channels of rats RBCs that were registered almost in all observation periods. Thus, after the single prolonged exposure, a significantly increased $P_{Ca2+-K+}$ value was recorded on Day 1, then it continued to rise, and on Day 5 the highest level of the Ca^{2+}-activated K^+-channels activity was registered in that group of animals. Further on, an essential attenuation of Ca^{2+}-dependent K^+-channels activity achieving levels lower than in Norm was observed on Day 10 followed with the significant increase of this index by Day 20 (Table 2).

Table 2. Activity of Ca^{2+}-activated K^+-channels (**$P_{Ca2+-K+}$**) of erythrocytes membranes on days 1, 5, 10 and 20 after rats exposure to 1800 MHz frequency RF-EMR (Norm: 55.44±0.79 x10^{-9} cm/sec)

Mode of exposure	Days after the exposure			
	1	5	10	20
Single	65.61±1.52*	75.30±1.70*	41.92±2.81*	69.81±0.19*
Fractional	56.58±1.18	39.08±0.17*	37.81±2.22*	63.07±0.24*

Note: *- p<0.05

At fractional mode of animal exposure a pronounced attenuation of Ca^{2+}-dependent K^+-channels activity was registered on Days 5 and 10. However, in the late period, i.e. on Day 20, statistically significant increase of $P_{Ca2+-K+}$ value was noted as compared to Norm (Table 2).

Thus, essential alterations in Ca^{2+}-activated K^+-channels activity of RBCs produced with 1800-MHz frequency RF-EMR at both schemes of influence on rats might testify to the possible changes in Ca^{2+} signaling pathways that might have an important impact on erythrocytes structural/functional properties and the life-span of the cells.

According to the results of the RBCs membrane potential measurements, on Day 1 post the single 2-hour acute exposure the hyperpolarization of erythrocytes membranes with a significant increase of E_m absolute value took place, after which a recovery to Norm on Days 5 and 10, and then significant decrease of E_m absolute value were observed on Day 20 (Table 3).

Table 3. Erythrocytes membrane potential (**E_m**) on Days 1, 5, 10, and 20 after the rats exposure to 1800 MHz frequency RF-EMR (Norm: -6.50±0.22 mV)

Mode of exposure	Days after the exposure			
	1	5	10	20
Single	-10.67±4.77*	-6.38±0.41	-7.08±0.42	-5.66±0.19*
Fractional	-7.15±0.39	-7.83±0.29*	-6.38±1.96	-4.51±0.19*

Note: *- p<0.05

After the fractional irradiation of animals, shifts in the RBCs membrane potential were also recorded as following: significant increase of E_m absolute value on Day 5 with recovery

to Norm on Day 10 and an expressed decrease of the E_m electronegativity on Day 20 as in case of use of the single 2-hour mode of rats exposure to RF-EMR (Table 3).

Determination of LPO indices revealed that this parameter also underwent changes due to the animal organism exposure to 1800 MHz frequency RF EMR (Table 4). Thus, a significant activation of LPO processes in erythrocytes membranes was revealed at all observation periods after the single 2-hour exposure, while alterations of this parameter had an undulating character post the fractional exposure viz., statistically significant increase of LPO_{er} level on Days 1 and 10 post the exposure with a remarkable decay on Days 5 and 20.

Table 4. Lipid peroxidation intensity in erythrocytes on Days 1, 5, 10, and 20 after the rats whole body exposure to RF-EMR with 1800 MHz frequency (Norm: 41.42±1.41 nanomoles MDA/mL eryth.mass)

Mode of exposure	Days after the exposure			
	1	5	10	20
Single	49.00±0.86*	48.26±0.73*	47.54±1.53*	46.95±0.10*
Fractional	47.85±2.47*	38.49±1.61	49.07±1.71*	39.77±3.10

Note: *- $p<0.05$

Our research revealed the biological response of erythrocyte membrane to 1800 MHz frequency RF-EMR in a form of alterations in functional properties after animal total body exposure. Moreover, the long-lasting character of the effects was detected. Data obtained suggest the following mechanism of biological activity of GSM-like RF EMR of 1800 MHz frequency: the cell membrane could be the site of interaction of low intensive RF EMR by altering the rate of calcium ion binding to enzyme and/or receptor sites. Any shifts in the electrochemical microenvironment of the cell can cause modifications in the structure of its electrified surface regions by changing the concentration of a specifically bound ions or dipoles that may be accompanied by alterations in the conformation of molecular entities such as lipids, proteins, and enzymes in the membrane structure.

Conclusion

Low intensive EMR with 1800 MHz frequency possesses an apparent biological activity and upon total influence on the organism facilitates development of effects manifested, in part, as alterations in functional characteristics of red blood cells membranes.

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