

S. Bajinyan, M. Malakyan,  
D. Yeghiazaryan, H. Aghjoyan,  
L.Vardevanyan

Scientific Centre of Radiation  
Medicine and Burns, Yerevan,  
Armenia

[labbio@web.am](mailto:labbio@web.am)

## EFFECTS OF LOW INTENSIVE 900-MHZ RF-EMR ON ANIMAL BLOOD INDICES AFTER SINGLE ACUTE OR FRACTIONAL TOTAL BODY EXPOSURE

**Abstract:** Functional indices of blood plasma and erythrocytes of white rats exposed to low intensity radio-frequency electromagnetic radiation (RF-EMR) were studied. Two modes of irradiation were used: a) 1-time action during 2 hours, i.e. single 2-hour acute exposure; b) fractional exposure to RF-EMR during 4 consecutive days for 0.5 hour daily. Sham-treated rats served as a Norm. On days 1, 5, 10 and 20 after the last exposure 5 rats per observation day were sacrificed and blood samples were drawn to determine intensity of lipid peroxidation in blood plasma and erythrocytes, integral antioxidant activity of water-soluble non-enzymatic antioxidants of rat blood plasma, erythrocyte membrane potential, K<sup>+</sup> permeability across the erythrocytes membranes, and the functional state of the Ca<sup>2+</sup>-activated K<sup>+</sup>-channels of erythrocytes. According to data obtained, 900-MHz RF-EMR caused statistically significant changes in the studied parameters both in early and late observation periods. Hence, the character and the dynamics of changes depended on the mode of irradiation.

Thus, a conclusion can be drawn that upon total influence on the organism low intensive EMR with 900 MHz facilitates development of long-lasting effect manifested as changes in functional characteristics of blood plasma and red blood cells.

**Key words:** 900-MHz, blood plasma, erythrocytes, membrane

## INTRODUCTION

Tremendous increase in the use of mobile phones brings forth growing concerns about the possible adverse effects of radio-frequency electromagnetic radiation (RF-EMR) emitted by these devices on human health. Numerous experimental and epidemiological data testify that the ever-growing exposure of living organisms to RF-EMR promote the occurrence of different disturbances in functional activity of cells and organs, which stimulate development of several sicknesses in humans [1, 2].

At present, there is an established occurrence of common manifestations of cell reactivity in response to the action of various agents that is stipulated by the existence of unique physical and chemical system of cell metabolism regulation, as well as by the uniformity of changes in structural-functional state of cell membranes [3]. It is supposed that the majority of biological effects of low intensity EMR are manifested not as a result of a direct action of non-ionizing radiation, but are mediated through this system of regulation involving, among others, alterations of the organism antioxidant status [4-7].

This study was designed to investigate membrane and biochemical effects of rats total body exposure to low intensity RF-EMR with 900-MHz frequency.

## MATERIALS AND METHODS

A total of 50 albino Wistar rats of 6 months age weighing between 180 g and 200 g were acclimated to the vivarium for at least 5 days before experimental manipulations are conducted. Rats were housed in plastic cages per 5 on cage allowed free access to food and water before treatments.

In order to perform irradiation rats of each experimental group were placed in well-ventilated plastic box (25 x 22 x 15 cm<sup>3</sup>) and exposed to whole body treatment with microwaves of 900-MHz frequency (7 μWt/cm<sup>2</sup> power density). A generating unit of X1-42 panoramic measuring instrument emitting microwaves with frequencies in the range of 0.1 – 1250 MHz and having 8-10 mWt output power served as a source of microwaves irradiation. Oscillator output was matched with 50-Ohmic cable. The generating unit was provided with output power regulator and had a capability with a high precision to establish the central frequency and the generation band in the entire range of frequency rearrangement in the digital display.

A fractal type Minkowski compact antenna (size: 11.7 x 12 cm<sup>2</sup>) of new generation with irradiation band within 850 – 960 MHz range served as an irradiator.

The resonance frequency of our antenna was designed so that the central frequency was equaled to

900 MHz. Amplification coefficient of antenna was 5.5 dB (~3.5-fold). At 3 dB level of the amplitude value the direction diagram was  $\pm 35^\circ$ . Subsequently, irradiation spatial angle is  $\sim 70^\circ$  that is corresponded to the irradiation area, i.e. to the object folding spatial angle. Since the antenna's size is sufficiently small, conditions for the remote area are quite acceptable.

Two modes of irradiation were used: a) 1-time action during 2 hours, i.e. single 2-hour acute exposure; b) fractional exposure to RF-EMR during 4 consecutive days for 0.5 hour daily. Sham-treated rats served as Control.

On days 1, 5, 10 and 20 after the last exposure 5 rats per observation day were sacrificed under light anesthesia with ethyl ether and blood samples were drawn to determine intensity of lipid peroxidation in blood plasma ( $LPO_{pi}$ ) and erythrocytes ( $LPO_{er}$ ), integral antioxidant activity of water-soluble non-enzymatic antioxidants (AOA WSNEAO) of rat blood plasma, erythrocyte membrane potential  $E_m$ ,  $K^+$  permeability across the erythrocytes membranes ( $P_K$ ), functional state of the  $Ca^{2+}$ -activated  $K^+$ -channels of erythrocytes ( $P_{Ca-K}$ ).

## RESULTS

Erythrocytes are a reliable and easily obtainable model to detect membrane perturbations; their own very simple internal structure depleted of nucleus and organelles offers an ideal environment not affected by complex and renewable buffer system, in which any cause-effect relationship can be clearly shown. Due to the unique bioelectrochemical properties cell membranes are considered as the most likely acceptors of microwave radiation. Cell activity and viability depend on the state of the cell membrane.

Membrane effects of total body exposure of rats to RF-EMR with 900-MHz frequency were evaluated analyzing the total permeability for  $K^+$  ions, including functional state of specific  $Ca^{2+}$ -activated  $K^+$ -channels of erythrocytes, as well as erythrocyte membrane potential as characteristic indices of erythrocyte membrane functional state.

According to data obtained, 900-MHz RF-EMR caused statistically significant changes in the studied parameters both in early and late observation periods. Hence, the character and the dynamics of changes depended on the mode of irradiation.

Thus, only on day 20 after fractional irradiation significant high levels of erythrocytes  $P_K$  and  $P_{Ca-K}$  were obtained, while in other study periods there were no essential changes of these indices (Tables 1 and 2).

In case of single long-lasting exposure of animals to 900-MHz EMR an enhanced total  $K^+$  outflow from erythrocytes was observed in the late periods that was maximally expressed on day 10. As to  $Ca^{2+}$ -depended  $K^+$ -channels of erythrocytes, the pronounced activity was observed on day 1 with certain attenuation on day

5, while at later period a significant exhaustion of  $P_{Ca-K}$  was noted.

**Table 1.**  $K^+$  permeability of erythrocytes on days 1, 5, 10 and 20 after whole body exposure of rats to RF-EMR with 900-MHz frequency (\*-  $P < 0.05$ )

Days	$P_{K^+}$ , Control: $2.35 \pm 0.15 \times 10^{-9} \text{ cm/sec}$	
	Single 2-hour exposure to 900- MHz RF-EMR	Fractional exposure to 900-MHz RF-EMR (4 days, 0.5 h/day)
1	$2.30 \pm 0.35$	$2.18 \pm 0.14$
5	$2.76 \pm 0.37$	$2.50 \pm 0.32$
10	$5.66 \pm 0.78^*$	$2.37 \pm 0.22$
20	$3.75 \pm 0.54^*$	$2.90 \pm 0.16^*$

**Table 2.** Activity of  $Ca^{2+}$ -depended  $K^+$ -channels of erythrocytes on Days 1, 5, 10 and 20 after whole body exposure of rats to microwave irradiation with 900-MHz frequency (\*-  $P < 0.05$ )

Days	$P_{Ca-K}$ , Control: $61.05 \pm 2.65 \times 10^{-9} \text{ cm/sec}$	
	Single 2-hour exposure to 900- MHz RF-EMR	Fractional exposure to 900-MHz RF-EMR (4 days, 0.5 h/day)
1	$195.00 \pm 18.20^*$	$71.92 \pm 7.49$
5	$96.15 \pm 10.21^*$	$82.12 \pm 9.71$
10	$41.6 \pm 13.40^*$	$65.49 \pm 7.24$
20	$31.92 \pm 9.16^*$	$80.30 \pm 6.49^*$

On day 1 post the single 2-hour acute exposure and on days 10 and 20 after fractional irradiation a hyperpolarization of erythrocytes membranes with a significant increase of  $E_m$  absolute value was recorded (Table 3)

**Table 3.** Membrane potential of erythrocytes on Days 1, 5, 10 and 20 after whole body exposure of rats to RF-EMR with 900-MHz frequency (\*-  $P < 0.05$ )

Days	$E_m$ , Control: $-7.62 \pm 0.61 \text{ mV}$	
	Single 2-hour exposure to 900- MHz RF-EMR	Fractional exposure to 900-MHz RF-EMR (4 days, 0.5 h/day)
1	$-10.08 \pm 0.55^*$	$-6.92 \pm 0.23$
5	$-6.09 \pm 0.35$	$-6.59 \pm 0.34$
10	$-7.17 \pm 0.86$	$-11.89 \pm 0.90^*$
20	$-6.99 \pm 0.88$	$-9.57 \pm 0.77^*$

Significant high indices of  $LPO_{er}$  were registered in animals in all periods of investigation either after the single prolonged or fractional exposure to 900-MHz EMR (Table 4).

**Table 4.** Lipid peroxidation intensity in erythrocytes on Days 1, 5, 10 and 20 after whole body exposure of rats to RF-EMR with 900-MHz frequency (\*- P<0.05)

Days	LPO <sub>er</sub> ,	
	Control: 32.44±0.70 nM MDA /mL er.	Fractional exposure to 900-MHz RF-EMR (4 days, 0.5 h/day)
1	57.01±2.05*	53.86±1.50*
5	58.87±1.30*	51.14±0.70*
10	52.56±0.45*	51.89±1.71*
20	45.49±1.67*	40.73±3.08*

On day 1 after 2-hour acute exposure an enhanced intensity of LPO<sub>pl</sub> was observed along with inhibited AOA WSNEAO. However, on the other observation days a significant decrease of LPO<sub>pl</sub> was accompanied with the pronounced elevation on AOA WSNEAO (Table 5).

**Table 5.** Lipid peroxidation intensity in plasma and integral antioxidant activity of water-soluble non-enzymatic antioxidants on Days 1, 5, 10 and 20 after 2-hour acute rats exposure to RF-EMR with 900-MHz frequency (\*- P<0.05)

Days	Single 2-hour exposure to 900 MHz RF-EMR	
	LPO <sub>pl</sub> , Control: 0.34±0.12 nM MDA /mL pl.	LPO <sub>pl</sub> , Control: 0.34±0.12 nM MDA /mL pl.
1	0.56±0.05*	0.56±0.05*
5	0.19±0.03*	0.19±0.03*
10	0.20±0.02*	0.20±0.02*
20	0.25±0.09	0.25±0.09

In case of rats fractional exposure to RF-EMR very low values of LPO<sub>pl</sub> indices were obtained on days 1 and 5 that was associated with the high AOA WSNEAO of blood plasma. (Table 6).

**Table 6.** Lipid peroxidation intensity in plasma and integral antioxidant activity of water-soluble non-enzymatic antioxidants on Days 1, 5, 10 and 20 after 2-hour acute rats exposure to RF-EMR with 900MHz frequency (\*- P<0.05)

Days	Fractional exposure to 900-MHz RF-EMR (4 days, 0.5 h/day)	
	LPO <sub>pl</sub> , Control: 0.34±0.12 nM MDA /mL pl.	LPO <sub>pl</sub> , Control: 0.34±0.12 nM MDA /mL pl.
1	0.013±0.002*	0.013±0.002*
5	0.030±0.004*	0.030±0.004*
10	0.52±0.03*	0.52±0.03*
20	0.37±0.05	0.37±0.05

Then, in the late periods an increase in LPO<sub>pl</sub> activity on day 10 and recovery of this parameter on day 20 were concurrent with the corresponding low level of

AOA WSNEAO on day 10 and normalization of LPO<sub>pl</sub> on day 20.

## CONCLUSION

A conclusion can be drawn that low intensive EMR with 900 MHz frequency possesses an apparent biological activity and upon total influence on the organism facilitates development of long-lasting effect manifested as changes in functional characteristics of blood plasma and red blood cells.

**Acknowledgement:** This research was performed in the frames of 11-1f295 project that is financially supported by the State Committee of Science of the Republic of Armenia.

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## BIOGRAPHY

**Margarita Malakyan** graduated from the Yerevan State University, Armenia in biophysics. She was awarded title of Doctor of Biology and the PhD by the Higher Certification Committee of the Ministry of Education and Science of Armenia. Dr Malakyan was a researcher at the Laboratory of pathophysiology and pharmacology in the Research Institute of Cardiology, Yerevan; head of the Laboratory of biophysics and radiobiology in the Scientific Centre of Radiation Medicine and Burn,



## EFEKTI RADIO FREKVENTNOG ELEKTROMAGNETNOG ZRAČENJA NISKOG INTENZITETA, FREKVENCE 900 MHz NA KRVNE INDEKSE ŽIVOTINJA, NAKON JEDNOKRATNE AKUTNE ILI PARCIJALNE TOTALNE EKSPOZICIJE

**S. Bajinyan, M. Malakyan, D. Yeghiazaryan, H. Aghjoyan, L.Vardevanyan**

**Rezime:** Ispitivani su funkcionalni indeksi krvne plazme i eritrocita belih miševa izloženih elektromagnetnom radio-frekventnom zračenju niskog intenziteta. Upotrebljavana su dva načina izlaganja polju: a) jednočasovno izlaganje tokom dvočasovnog perioda, odnosno dvočasovno neprekidno izlaganje; b) parcijalno izlaganje radio-frekventnom elektromagnetnom zračenju u toku 4 uzastopna dana, po 0,5h dnevno. Netretirani miševi su uzeti kao referentna grupa. Nakon jednog, pet, deset i dvadeset dana nakon poslednjeg izlaganja zračenju, 5 miševa po danu observacije je žrtvovano, a krv uzorkovana kako bi se utvrdio intenzitet lipidne peroksidacije u krvnoj plazmi i eritrociti, integralna antioksidantna aktivnost u vodi rastvorljivih neenzimskih antioksidanata, potencijal membrane eritrocita,  $K^+$  permeabilnost duž eritrocitne membrane i funkcionalni status  $Ca^{2+}$  aktiviranih  $K^+$  kanala eritrocita. Prema rezultatima istraživanja, radio-frekventno elektromagnetno zračenje frekvence 900 MHz izazvalo je statistički značajne promene posmatranih parametara, kako u ranim, tako i u kasnim observacionim periodima. Prema tome, karakter i dinamika promena zavisili su od načina izlaganja polju.

U skladu sa prethodnim, može se izvući zaključak da, u sklopu totalnih uticaja na organizam, elektromagnetno zračenje niskog intenziteta i frekvence 900 MHz pospešuje razvoj dugoročnih efekata koji se manifestuju kao promene funkcionalnih karakteristika krvne plazme i crvenih krvnih zrnaca.

**Ključne reči:** 900-MHz, krvna plazma, eritrociti, membrana.