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Bioeffects of electromagnetic irradiation on blood of rats

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Electromagnetic radiation (EMR) generated by various devices (radars, mobile phones, medical equipment) causes biochemical changes in the blood of humans and animals. Study of the effects of 460 MHz EMR on the blood levels of lipid peroxidation, total antioxidant activity (TAA) and reduced glutathione in the rats. Rats weighing 250‒300g were subjected to the whole body exposure with 460 MHz EMR at the power density of 30 µW/cm^2 for 4 weeks, 6 days/week, 20 min/day. For this intensity of radiation, value of specific absorption rate (SAR) was estimated as 15 mW/kg. A dramatic increase in plasma MDA concentration was observed in the 1st week of exposure, which gradually decreased to a level slightly higher than that of control for other exposure periods. Erythrocyte MDA concentration was higher than that for the control animals for the 2nd and 4th weeks. TAA changes in the plasma and erythrocytes were little and statistically insignificant. Reduced glutathione concentration was found to decrease significantly by the end of the 4th week of exposure (p<0.01). The results indicate that free radical processes and antioxidant protection in the cells are involved in the mechanism of bioeffects of electromagnetic radiation in microwaves frequency range.

Keywords: *Electromagnetic radiation, plasma, erythrocytes, lipid peroxidation, antioxidant activity, reduced glutathione*

INTRODUCTION

In the modern world, the density of electromagnetic radiation (EMR) in the environment is growing steadily. This is due to the constant growth in the use of technical means that generate electromagnetic radiation in a variety of areas of human activity. Today, it is quite impossible to imagine the life of every person without devices and equipment of communication by the use of computer technology, EMR sources are intensively applied in medicine, military and household appliances, as well. The emergence of mobile communications has brought the problem of electromagnetic pollution of the environment to new aspect, i.e., now almost every person carries “their own individual electromagnetic environment”. Therefore, the study of the influence of such close to the human body sources is of great interest for the researchers, involved in the identification of biological effects of electromagnetic radiation and elucidation of their molecular mechanisms.

One of important areas of researches in electromagnetic biology is the study of the effect on the body of microwave electromagnetic irradiation. In particular, the sources of microwave radiation are mobile phones and their base stations, physiotherapy units and radars. They radiate in the frequency range corresponding to decimeter, centimeter and millimeter wavelengths. There are a lot of scientific works that are devoted to the study of changes in individual organs and tissues exposed to microwave radiation. The general focus of these studies is to identify the effect of low - intensity microwave irradiation on oxidative metabolism in different organs and tissues (Kivrak et al., 2017). Such processes as lipid peroxidation, antioxidant protective reactions in the brain, heart, liver, kidney and blood cells of the experimental animals, exposed to microwaves, were studied (Oktem et al., 2005; Elhag et al., 2007; Moussa, 2009; Megha et al., 2012; Alghamdi et al., 2012; Abbasova, 2015; Bodera et al., 2015). An interesting fact is that non - thermal effects of microwave radiation on pro- and antioxi-

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dant parameters of various tissues have been found. In particular, we have shown that exposure of the rats to microwave irradiation (from a mobile phone) up to 4 weeks for 20 min a day causes shifts in the intensity of tissue respiration of the brain structures, correlating with changes in the rate of formation of reactive oxygen species in mitochondria and lipid peroxidation intensity (Gadzhiev et al., 2016). In our other experiments, we observed the intensity-dependent pro- or antioxidant effects of EMR at 460 MHz in the lens of the eyes in the rats (Musaev et al., 2009). This effect was observed in the range of non-thermal or low intensity.

As for the effect of EMR in microwave range on the antioxidant agents of the cell, Yurekli et al. (Yurekli et al., 2006) showed widespread biological effects at a power density of 3.67 W/m^2 with specific absorption rate of 1-3mW/kg in reduced glutathione concentration in the rats. Other studies have indicated that irradiation induces reactive oxygen species (ROS), which play an important role in radiation damage of the cells (Cemek et al, 2006). The same study showed reduced glutathione (GSH) level as having antiperoxidative effect on different tissues and a scavenger effect on ROS.

The most adequate object for the study of the effects of EMR on the living organism is the blood, which, on one hand, circulates between many organs and tissues, exchanges the products of metabolism between them, including those products that arise due to the interaction of cellular structures with EMR (oxygen radicals, products of lipid peroxidation, carbonyl derivatives of proteins etc.). On the other hand, due to the high water content, the blood absorbs a significant part of electromagnetic radiation energy and transmits it to the membrane structures of blood cells and proteins of plasma. In this context, blood can serve as a source for determining the markers of EMR action on the body at the level of the oxidant-antioxidant relationship system. It is of great interest to identify qualitative and quantitative differences in this system subjected to the action of microwave EMR of intensity in the non-thermal range. This paper aims to study the concentration of lipid peroxidation product malondialdehyde and total antioxidant activity of plasma and erythrocytes in the rats subjected to whole body exposure by 460 MHz EMR at a relatively high power density. Reduced glutathione content was also determined in order to evaluate antioxidant status in the blood samples of exposed rats.

MATERIALS AND METHODS

The experimental protocol was approved by the Local Ethics Committee for Animal Experimentation (28.11.2012, protocol No.18).

Experiments were conducted on the male Wistar albino rats weighing 250−300 g, kept in normal vivarium conditions. The animals were divided into 2 groups of 10 rats in each. One group was a control group (Con), the other - for that of (relatively) high-intensity (HIE). The apparatus "Volna-2" (manufactured in Russia) was used for the whole body exposure of animals. This device is commonly used for physiotherapy in clinics, and presents a tube generator of EMR, designed for therapeutic purposes to carry out a dosed effect on the patient by an electromagnetic field with the frequency of 460 MHz (±1%) in the decimeter wave range (wavelength 65 cm). The experiments were carried out at a relatively high intensity with power density of 30 $\mu W/cm^2$ (the output power of apparatus - 60 W). Averaged over the whole body values of SAR (Special Absorption Rate) for this intensity of irradiation were estimated by temperature change calculation and obtained, respectively, 15 mW/kg. The control group of animals was exposed to sham exposure under the same conditions with the radiation source turned off. Intact animals were not involved in the study due to the lack of significant differences in measured blood parameters between them and sham group of animals in preliminary experiments. Expositions for both experimental groups were 20 min a day, 6 days a week. The experiments were set separately for 1, 2, 3, and 4-week exposure of the animals.

Lipid peroxidation was estimated by measuring the concentration of the colored complex formed by malondialdehyde with externally-added thiobarbituric acid (TBA), according to Andreyeva et al. (1988) in plasma and Suplotov et al. (1986) in erythrocytes.

The level of reduced glutathione in hemolysed blood was assessed by Ellman method (Ellman, 1999). The main principle of the method is based on the formation of a color combination as a result of interaction of the Ellman reactive (5,5 ditiobisnitrobenzoic acid) with SH groups.

The total antioxidant activity in plasma and erythrocytes was determined by the method of Goryachkovsky (1996) in which the activity was estimated by the degree of inhibition of twin-80 oxidation to malondialdehyde by ascorbic acid-ferrous system.

SPSS for Windows version 22.0 package program was used for statistical analyses of the data. Shapiro-Wilk test was used to check whether the variables for studied groups fit normal distribution. Differences between control and experimental measurements were tested using paired samples Student's criterion. Mean ± standard error values were given as the descriptive statistics and $p<0.05$ was accepted as the statistically significant value.

RESULTS AND DISCUSSION

The concentration of plasma MDA increases dramatically after a week of exposure to relatively high intensity of irradiation (Table). The excess in concentration relative to the control animals is 96.5% at $p<0.001$ by the end of first week. The MDA level gradually decreases following the increase in duration of exposure to irradiation, and after 4-week exposure reaches a level exceeding the control level by 20% ($p<0.05$).

As for the red blood cells, yet after 2 weeks of exposure the MDA level in them becomes quite high, but not stable. To the ends of the 2nd and the 4th weeks, the increases in MDA levels reached 37.8% and 32.9%, correspondingly. At 3-week exposure of irradiation, a small excess of MDA level relative to the control group has no statistical reliability.

The level of reduced glutathione (GSH) in hemolysed blood in the rats, exposed to EMR, varies depending on the duration of radiation exposure. After the first week of exposure, the level of reduced glutathione decreases compared to the control group. Despite the absence of significant changes in the level of glutathione for 2^{nd} week of exposure, after 3 and 4 weeks of exposure, the level of glutathione is significantly lower than in the control animals. At the end of 4 weeks of exposure, the reduction in reduced glutathione levels is approximately 50% of the control value (1.03 μmol/l vs. 2.0 μmol/l in the control). Thus, with an increase in the duration of exposure to radiation, the level of the constituent element of the blood antioxidant system, glutathione, decreases, and this indicates a weakening of the organism's antioxidant defense system.

Exposure of the rats to EMR at a relatively high intensity does not lead to significant changes in the level of TAA in plasma and red blood cells. Thus, the level of plasma TAA showed a slight decrease (12%) without 95% confidence of probability for only 2 week duration of exposure from those of all studied time segments. TAA of erythrocytes have no obvious signs of dependence on the duration of irradiation.

The decrease in the concentration of GSH in the blood, when the organism is exposed to microwave radiation, seems to be associated with a decrease in its synthesis in the liver, as well as its increased expenditure during the intensification of free-radical processes throughout the organism (Tolpygina, 2012). GSH can interact directly with free radicals, neutralizing them, but its main antioxidant and other functions are realized through enzyme systems. Damage to enzymes such as glutathione reductase, glutathione peroxidase, glutathione transferase, SOD, catalase, and some others, in pathologies or under the influence of external factors, complicates or paralyzes the enzymatic pathway of removing toxic compounds-oxidants of different nature from cells and tissues. In this aspect, a decrease in GSH activity in the blood under the influence of microwave irradiation, which has an oxidative character, on the background of minor changes in total antioxidant activity of plasma and red blood cells, may occur.

There is a number of works, pointing out to the increase in the level of free radical production in organs and tissues under the effect of microwave EMR of low intensity, taking place, for example, when using mobile phones (Challis, 2005). EMRs, no matter where they occur in the frequency spectrum, are reported to cause a rise in the levels of oxygen free radicals in experimental environments in the plants (Ursache et al., 2009) and humans (Georgiou, 2010).

Table. MDA and reduced glutathione concentrations in the blood of rats exposed to EMR 460 MHz at relatively high intensity (power density of irradiation - 30 μW/cm^2, averaged over the whole body value of SAR – 15 mW/kg)

Parameters	Control	Exposed			
		1 week	2 weeks	3 weeks	4 weeks
Plasma MDA, nmol/l	7.89±0.76	15.50±0.98**	10.10±1.10*	9.63±0.98*	9.47±0.80*
Erythrocyte MDA, nmol/l	11.50±1.50	10,60±0.82	15.80±1.34*	11.95±0.30	15.32±0.83*
Reduced glutathione, μmol/l	2.00±0.50	1.60±0.40*	2.10±0.50	1.70±0.30	1.03±0.10**

* - p<0.05 and ** - p<0.01- compared to the control group

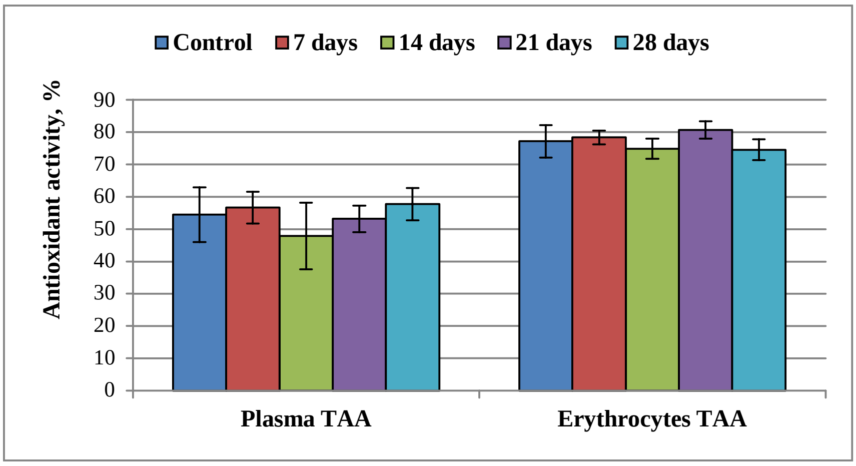

Fig. TAA levels in plasma and erythrosytes in rats exposed to EMR 460 MHz at relatively high intensity.

In the work of Elhag et al. (Elhag et al., 2007), it is indicated that EMR of cellular phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation and by changing the antioxidant activities of human blood that is leading to oxidative stress. Using EMR of 900 MHz (GSM standard), they observed an increase in the MDA concentration in the plasma of the exposed rats. At the same time, there was a decrease in the concentrations of low molecular weight antioxidants, ascorbic acid, vitamin E, reduced glutathione, as well as the activity of antioxidant enzymes catalase and superoxide dismutase. In another work (Moussa, 2009), microwave irradiation (3.5 GHz) applied for exposure of male rats also caused a significant increase in the plasma lipid peroxidation marker (MDA) while a significant decrease in glutathione concentration was observed. Results consequently suggest that the redox potential of glutathione (GSH) and nicotinamide adenine dinucleotide (NADH/NAD) were disturbed as a result of the irradiation exposure.

Bodera and others in their studies on microwave range EMR effects (5 days, 15 min/day, 1800 MHz) also showed that the levels of lipid peroxidation increase in the blood, kidneys and brain, and the total antioxidant activity of the blood at the same time becomes lower than that in the control animals (Bodera et al., 2015).

The results obtained by Moustafa et al. (2001) showed that the plasma level of lipid peroxidation significantly increased after acute exposure (up to 4 h) to the mobile phone EMR. At the same time, the activities of superoxide dismutase and glutathione peroxidase in erythrocytes showed significant reduction while the activity of catalase did not.

As shown in the work of Kumar et al. (Kumar et al., 2010), microwave radiation of extremely high frequency (10 and 50 GHz) induces significant increase in ROS production in the chronically exposed rats. The activities of serum superoxide dismutase and glutathione peroxidase decreased, and thus, the factor reduces total antioxidant capacity in the blood.

The Fenton reaction is a catalytic process that converts hydrogen peroxide, a product of mitochondrial oxidative respiration, into a highly toxic hydroxyl free radical. Some studies have supposed that EMR has another mechanism through the Fenton reaction, suggesting that it promotes free radical activity in the cells (Lai et al., 2004; Aydin et al., 2011).

There are some studies on the effect of microwave EMR on the parameters of iron in blood serum, which may indicate indirectly to the participation of Fenton's reaction in the mechanisms of free radical realization of microwave radiation in the living systems. In the work of Chetkin et al. (2017), it was shown that although chronic exposure of the rats to mobile phone irradiation does not lead to a change in the level of serum iron and ferritin, but it negatively affects both unsaturated and total iron capacities of the serum. In our previous study (unpublished result), where we used the same irradiation (460 MHz) for exposure of the rats, as in this study, we also found changes in the values of serum iron, in both unsaturated and total iron binding capacities of serum.

The biological effect of exposure to microwave EMR is a subject of particular research interest. The results of many studies not only clearly demonstrate that EMR exposure triggers oxidative stress in various tissues, but also that it causes significant changes in the levels of blood oxidant and antioxidant markers. Apparently, the risk of oxidative stress in various organs, including blood, due to electromagnetic radiation should be taken into account (Kivrak et al., 2017; Dasdag et al., 2016). However, the realization of these risks is likely to be determined by the duration of exposure to EMR and power flux density of irradiation. The risks of various disorders in the body, associated with the influence of EMR, probably depend also on the functional state of organism and additional environmental factors (Bodera et al., 2015; Yakymenko et. al., 2016; Abdolmaleki et al., 2012).

Thus, the results of our research and the data of other authors, which we have cited, allow us to expand the understanding of changes in lipid peroxidation processes and activity of antioxidant system to the effort of identification of interaction between peroxidation processes and the activation mexanism of these processes by EMR.

It seems that the generalization of the obtained experimental results can serve as a promotion for the development of methodological recommendations for primary aids in occurrence of emergency situations, associated with electromagnetic exposure.

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Elektromaqnit şüalanmasının siçovulların qanında bioeffektləri

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Müxtəlif elektrik avadanlıqlarının və cihazların (radarlar, mobil telefonlar, tibbi avadanlıqlar) yaratdığı elektromaqnit şüalanmaları (EMŞ) insanların və heyvanların qanında biokimyəvi dəyişikliklərə səbəb olur. EMŞ-nın müxtəlif orqan və toxumalara tezlik və amplitud (intensivlik) diapazonundan asılı olaraq göstərdiyi təsirin öyrənilməsi elektromaqnit biologiyasının mühüm tədqiqat məsələlərindəndir və bununla əlaqədar olaraq insanların həyatına daha dərindən nüfuz etmiş desimetr diapazonlu EMŞ-nın bioeffektinin üzə çıxarılması məqsədi qarşıya qoyulmuşdur. Model eksperimentdə bu diapazona daxil olan 460 MHz EMŞ-nın siçovulların qanında lipid peroksidləşməsinin, ümumi antioksidant aktivliyinin (ÜAA) və reduksiya olunmuş qlütationun səviyyələrinə təsiri öyrənilmişdir. Çəkisi 250−300q olan siçovullardan 4 qrup (hər qrupda 10 baş) uyğun olaraq 1, 2, 3 və 4 həftə müddətində, həftədə 6 gün, gündə 20 dəqiqə 460 MHz EMŞ ilə xroniki şüalandırılmışlar. Şüalanma kamerasında enerji selinin sıxlığı 30 $\mu W/sm^2$ olmuşdur və şüalanmanın bu intensivliyi üçün siçovulların bütün bədəninə ortalanaraq hesablanmış xüsusi udulma gücü (SAR – specific absorbtion rate) 15 mVt/kq civarında qiymətləndirilmişdir. 4 nəzarət qrupu (hərəsində 10 baş) eyni prosedurları 1, 2, 3 və 4 həftə müddətində EMŞ mənbəyinin söndürülmüş vəziyyətində keçmişlər. 1 həftə şüalanan heyvanların qan plazmasında lipid peroksidləşməsinin məhsulu malondiladehidinin (MDA) qatılığı nəzarət qrupu ilə müqayisədə kəskin artıma ($p<0,01$) məruz qalır. Şüalanmanın sonrakı həftələrində plazmada MDA-nin qatılığının tədricən kontroldan bir qədər yüksək səviyyəyə ($p<0,05$) düşməsi müşahidə olunur. 2 və 4 həftə şüalanmış heyvanlarda eritrositlərdə MDA-nin qatılığı nəzarət heyvanlarına nisbətən daha yüksək səviyyə ($p<0,05$) göstərmişdir. Plazma və eritrositlərdə ÜAA-nin dəyişmələri kiçikdir və statistik etibarlı deyil. Reduksiya olunmuş qlütationun qatılığının şüalanmanın 4-cü həftəsinin sonuna əhəmiyyətli dərəcədə azalması aşkar edilmişdir ($p<0,01$). Nəticələr göstərir ki, hüceyrələrdə sərbəst radikal peroksidləşmə prosesləri və antioksidant müdafiə elementləri mikrodalğa tezlik diapazonunda elektromaqnit şüalanmasının bioeffeklərinin mexanizmində iştirak edir.

Açar sözlər: *Elektromaqnit şüalanması, plazma, eritrositlər, lipid peroksidləşməsi, antioksidant fəallığı, reduksiya olunmuş qlütation*

Биоэффекты электромагнитного излучения в крови у крыс

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Электромагнитное излучение (ЭМИ), генерируемое различным электрооборудованием и приборами (радарами, сотовыми телефонами, медицинским оборудованием), вызывает биохимические изменения в крови людей и животных. Изучение влияния ЭМИ на органы и ткани, в зависимости от диапазона частот и амплитуд, является одним из принципиальных вопросов электромагнитной биологии, в связи с чем, выявление биоэффектов ЭМИ дециметрового диапазона, проникающего наиболее глубоко в жизнедеятельность современного человека, встает как первостепенная задача для исследования. В модельном эксперименте представленной работы изучалось влияние ЭМИ, равного 460 МГц и входящего в данный диапазон частот, на уровни перекисного окисления липидов, общей антиоксидантной активности (ОАА) и восстановленного глутатиона в крови у крыс. Из крыс, весом 250-300 г, были созданы 4 группы (по 10 голов в каждой группе), которые подвергались хроническому облучению в течение 1-й, 2-х, 3-х и 4-х недель (6 дней в неделю по 20 минут в день) соответственно при частоте 460 МГц. В камере для облучения плотность потока мощности (интенсивность облучения) составляла 30 мкВт/см^2, удельная скорость поглощения (SAR - specific absorbtion rate), усредненная по всему телу животного, при данной интенсивности облучения была оценена на уровне 15 мВт/кг. 4 контрольные группы (по 10 голов в каждой) проходили те же процедуры в течение 1-й, 2-х, 3-х и 4-х недель при выключенном аппарате источника ЭМИ. Концентрация продукта перекисного окисления липидов малонового диальдегида (МДА) в плазме крови животных, облученных в течение 1 недели, была значительно повышена ($p<0,01$) по сравнению с контрольной группой. В последующие недели облучения концентрация МДА в плазме постепенно снижалась до уровня, несколько превышающего уровень контроля ($p<0,05$). Концентрация МДА в эритроцитах у крыс, облученных в течение 2-х и 4-х недель, оказалась достоверно выше, чем у контрольных животных ($p<0,05$). Изменения ОАА в плазме и эритроцитах были небольшими и статистически недостоверными. Обнаружено значительное уменьшение содержания восстановленного глутатиона в плазме к концу 4-й недели облучения ($p<0,01$). Результаты показывают, что процессы свободнорадикального перекисного окисления и элементы антиоксидантной защиты в клетках участвуют в механизме биоэффектов электромагнитного излучения в диапазоне микроволновых частот.

Ключевые слова: *Электромагнитное излучение, плазма, эритроциты, перекисное окисление липидов, антиоксидантная активность, восстановленный глутатион*