International Scientific Journal
Medical and Biological Sciences
http://bioscience.scientific-journal.com

Study of Changes of Protein Carbonyl Content and Lipid Peroxidation Product in Blood of Rats Exposed to Decimeter Electromagnetic Radiation (460MHz)

M.T. Abbasova, A.M. Gadzhiev
A.I.Karayev Institute of Physiology of Azerbaijan National Academy of Sciences
Baku, Azerbaijan Republic
biokimya_65@mail.ru

***Abstract* —** Most used in physiotherapy physical factors has an electromagnetic nature. Initially, the therapeutic effect of electromagnetic radiation in a wide range of frequencies associated with activation of metabolic processes in the exposed tissues. The most highly-absorbing ultra high frequency (UHF) energy of tissues are the blood, lymph, parenchymatous tissue, muscles. One of the most important factors in the mechanism of biological effects of electromagnetic radiation may be their effect on free radical processes. This mechanism may be mediated by a free radical chain process including lipid peroxidation (LPO). The effect of free radicals on different types of proteins leads to complicated modifications in the structure of the protein molecule and thus change its physico-chemical and biological properties. In this paper, we studied the changes in the protein carbonyl levels and lipid peroxidation in blood of rats chronically exposed to 460 MHz electromagnetic radiation up to 14 days (daily for 20 minutes at a power density 30 mW/cm^2 and 10 mW/cm^2). The data obtained have shown that the content of Carbonyl derivatives of proteins in the plasma and blood serum is increased compared with control animals with high intensity, it is more pronounced in the serum. Also the ratio of Carbonyl derivatives in the serum and plasma is more dramatic for irradiated organism, and in low intensity decreases compared to the control. A content of malondialdehyde in high radiation intensity is increased compared to control, and at lower radiation intensity is decreased.

Keywords—protein carbonyl, lipid peroxidation, malondialdehyde, electromagnetic radiation, plasma, serum

I. Introduction

Study of the biological effects of non-ionizing electromagnetic radiation (EMR) is still the focus of researchers. The most important aspect of the research is to elucidate the mechanisms of action of a living organism of low-energy radiation, although the reasons for the use of EMR for hygiene, medical procedures and diagnostics are also the driving force of this research.

Implementation of the biological action of electromagnetic radiation in the microwave range through its influence on free radical processes is now considered one of the possible mechanisms [1-3]. Lipid peroxidation (LPO), involved in the regulation of these processes antioxidant defense elements in various organs and tissues affected by microwave irradiation. Previously, a number of studies involving the authors of this article have provided evidence that the EMR 460 MHz with whole body irradiation causes changes in the redox balance in the different structures of the brain and eyes [3, 11, 18, 19]. Moreover, these changes (the concentration of malondialdehyde LPO product, reduced thiols antioxidant activity of some enzymes) depending on the radiation intensity may exhibit as a prooxidant and antioxidant tendency [3, 4].

It is known that the formation of carbonyl derivatives of the proteins used as a marker of oxidative stress phenomena. Protein carbonyls have a long period of decay compared with the products of lipid peroxidation, and makes them more promising marker of the intensity of free radical oxidation [5, 6]. Recent advances in proteomics and mass spectrometry allow the identification of carbonyl derivatives of proteins in the blood and other tissues in various diseases and pathologies.

In this paper, we studied the changes in the protein carbonyl level and lipid peroxidation product in blood of rats chronically exposed to 460 MHz electromagnetic radiation. The main objective of the study was to get the facts about the oxidative nature of biological effect of decimeter range electromagnetic radiation.

II. Methods

Studies were carried out on Wistar albino rats weighing 250-300 g with contained in ordinary vivarium conditions. The experiment was conducted in compliance with ethical norms and rules of work with laboratory animals. Animals were randomly divided into experimental and control groups. Before the experiments with irradiation all animals within a week to adapt to the conditions of detention during the experiment. Experimental group of animals was irradiated EMR UHF (460 MHz) using the apparatus "Volna-2" (made in Russia) in a metal cylindrical chamber. The control animals were sham irradiated at the switched off apparatus. Irradiation

was carried out daily for 20 min to 14 day period. It has been implemented two modes of exposure: a relatively high-intensity irradiation at power density of 30 mW/cm^2 and a relatively low-intensity exposure - at 10 mW/cm^2. For study, it has been used a common blood collected at decapitation of rats, from which the plasma and serum were separated. Trisodium citrate solution of 3.8% (mass/volume) was used as anticoagulant. Proportion of blood and sodium citrate was was 1:9.

Protein oxidation was evaluated by measuring carbonyl formation using 2,4-dinitrophenylhydrazine (2,4-DNPH) as a reagent, according to Levine et al. (1990) [8], with some modifications described in work of Dubinina et al. (1995) [6]. The principle of the method based on the reaction of the oxidized amino acid residue of protein with 2,4-DNPH, which results in formation of 2,4-dinitrophenylhydrazone derivatives. The absorbance of dinitrophenylhydrazone derivatives in the samples was recorded at a wavelength of 370 nm. Carbonyl derivatives content was calculated by using a molar extinction coefficient - 22000M^{-1}cm^{-1}.

1 ml of 10 mM 2.4-DNPH with 2 N HCl was added to 0.1 ml plasma (or serum). The samples were incubated within an hour, vortexing every 15 min. Protein was precipitated with 1 ml of 20% trichloroacetic acid, followed by centrifugation at 3000 g for 20 minutes. The pellets were washed to remove excess DNHP and lipids with mixture of ethanol - ethyl acetate (1: 1). After drying, 3 ml urea was added to samples and boiled in a water bath for 10 minutes. Optical density of samples was registered by spectrophotometer "Spekol-221".

Content of malondialdehyde (MDA) determined by the method described in Andreeva et al. (1987) [7]. 0.3 ml of fresh blood serum or plasma without hemolysis was taken for analysis. 3 ml 1% phosphoric acid (pH is important to check, the density of the solution corresponds to 1.004 g/ml), 0.6 ml 1% TBA (stored in a dark bank with moderate heating to dissolve) and 0.1 ml of ferrous sulfate (28 mg ·FeSO4·7 H_2O in 10 ml of distilled water) were added to samples (serum or plasma). Test tubes were pplaced in a boiling water bath for 1 hour. Then, the tubes were cooled with cold water and after adding of 4 ml butanol, were carefully mixed and centrifuged 10 min at 3000 rev/min. Measure the absorbance of the upper phase conducted at a wavelength of 535 nm (E_{ex}) against butanol. Calculation of the content of products that react with TBA, performed taking into account the molar extinction coefficient of MDA $1.56 \cdot 10^5$ mol · sm^{-1}.

$$A = \frac{E_{ex} \cdot 10^6 \cdot 4ml}{1{,}56 \cdot 10^5 \cdot 0{,}3ml} = E_{ex} \cdot 85{,}47$$

where A-content of MDA (μmol/l or nmol/ml), 4 ml of butanol phases, 0.3 ml of serum.

Statistical analysis was made using program Microsoft Excel and significance of means' difference was evaluated using paired Student T-test.

III. Results and discussion

In the Fig. 1, concentrations of protein carbonyl groups in plasma and serum have been presented for both control and irradiated rats for the experiment with relatively high intensity exposure (30 mW/cm^2). As shown, the serum level of protein carbonyls for irradiated rats (27.3±5.2 nmol/ml) was consistently higher than that for control rats (12.4±2.7 nmol/ml); the difference was more than 100% at the significance $p<0.05$.

However, no significant difference in the levels of protein carbonyls in plasma had been observed between irradiated and control rats (3.41±0.74 and 2.95±0.47 nmol/ml, respectively, $p>0.05$).

The exposure for 14 days at a relatively low intensity (10 mW/cm^2) led to a result different from the high intensity of irradiation (Fig.2). In this case, the level of protein carbonyl in plasma is significantly below the irradiated rats compared to controls. Plasma carbonyl contents for control and irradiated rats had levels 11.5±2.7 and 5.3±2.0 nmol/ml, respectively, and decrease was 54% ($p<0.05$).

There was no significant change in serum protein carbonyl content among treatment groups in low intensity experiment. Here, control and irradiated groups had practically the same serum levels, about 30 nmol/ml.

The high intensity exposure to 460 MHz EMR for 14 days resulted in a significant increase in plasma lipid peroxidation in rats. Plasma MDA content for control and irradiated rats showed base levels of 6.2±1.3 and 24.5±10.0 nmol/ml, respectively, and approximately a 4-fold increase was occurred (Fig.3).

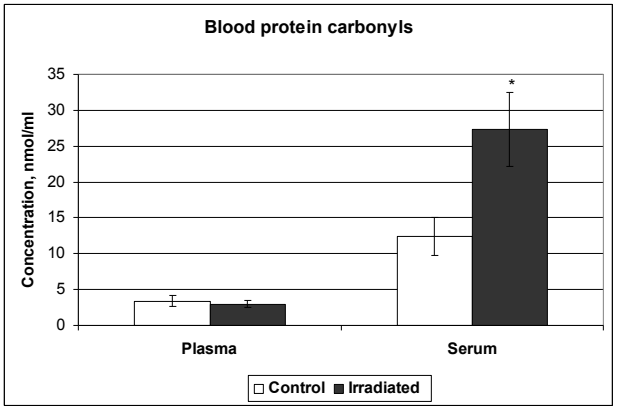

*Fig.1. Protein carbonyl contents in blood plasma and serum of rats exposed to 460 MHz radiation for 14 days 20 min a day at the power density 30 mW/cm^2 (relatively high intensity irradiation). Each bar represents mean ± SE (n – 6 rats). * P < 0.05 irradiated vs. control group.*

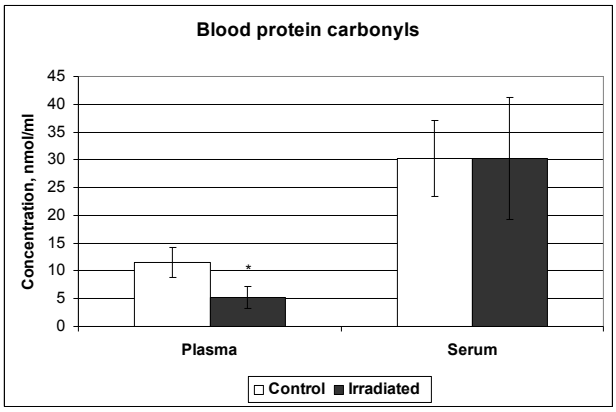

*Fig.2. Protein carbonyl contents in blood plasma and serum of rats exposed to 460 MHz radiation for 14 days 20 min a day at the power density 10 mW/cm^2 (relatively low intensity irradiation). Each bar represents mean ± SE (n – 6 rats). * P < 0.05 irradiated vs. control group.*

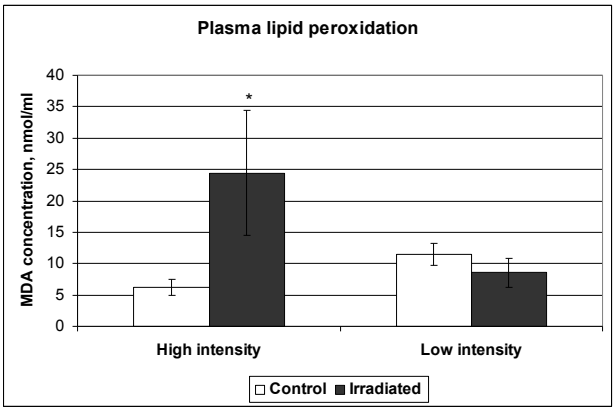

*Fig.3. Lipid peroxidation product malondialdehyde content in blood plasma of rats exposed to 460 MHz radiation for 14 days 20 min a day at high and low intensity exposure, relatively, at power densities 30 and 10 mW/cm^2. Each bar represents mean ± SE (n – 6 rats). * P < 0.05 irradiated vs. control group.*

Relatively low intensity exposure to 460 MHz EMR for 14 days was not demonstrated increase in plasma lipid peroxidation in rats. Contrary, rats exposed to relatively low-intensity irradiation tended to have lower MDA level (8.6±2.3 nmol/l) in plasma than that for control ones (11.5±1.7 nmol/l) but the difference 25% was not significant (p>0.05).

It is interesting that the ratio between serum and plasma protein carbonyl contents was significantly changed by exposure of rats to 460 MHz radiation. If this ratio was evaluated in the range of 3-4 for control animals, it was dramatically increased to range of 6-9 for irradiated ones. Relatively high intensity exposure resulted in higher ratio between serum and plasma protein carbonyl contents.

Our study has provided some interesting and significant data. First, the study supports the argumentation in favor of the oxidative nature of non-ionizing electromagnetic radiation effects on living systems. In other words, the implementation of the biological effect of microwave radiation can occur through free radical oxidation of lipids and proteins. Second, the chronic effect of electromagnetic radiation exposure on blood and probably other organs can have both prooxidant and antioxidant character depending on the intensity of exposure.

Previously, it has been shown that chronic exposure to EMR (GSM band) leads to the accumulation of lipid peroxidation products, decrease in the activity of SOD, glutathione peroxidase, and catalase while increasing xanthine oxidase and adenosine deaminase activity in the tissues of the brain and other organs of animals [9, 10]. The changes in lipid hydroperoxides concentration was observed in plasma of rats exposed to EMR 905 MHz generated by mobile phone [11]. Musaev et al. [12] revealed changes in intensity of ascorbate – and NADPH-depended induced lipid peroxidation in some brain and eye structures in rats exposed to microwaves 460 MHz. It has been shown that high intensity stimulated basal lipid peroxidation but suppressed activity of lipid peroxidation-inducing systems. According to the same work, relatively low irradiation intensity leads to activation of induced lipid peroxidation system, which is accompanied by a synchronous activation of the antioxidant system for normal redox balance of the cells. In the work of Gadzhiev and coworkers (2005) [13], thiol defense system of different structures of both eye and brain in rats was studied and pro- or antioxidant tendencies in changes of reduced thiols content were identified for, respectively, high and low intensity exposure by decimeter radiation.

Apparently, similar to the results of the above studies, 460 MHz EMR prooxidant effect was observed in our study on blood of rats exposed to high intensity irradiation at power density of 30 mW/cm^2. The concentration of the carbonyl derivatives of proteins in serum was increased by several times, however, the plasma concentration remained nearly unchanged.

This may indicate that the main contribution to the oxidative modification of proteins gives fibrinogen. It is well known that fibrinogen is more oxidizable protein than other major plasma proteins [14] Because of much greater concentration, fibrinogen may be considered an important antioxidant that protects the less oxidizable proteins or protein-lipid complexes. The loss of aggregation ability due to oxidative modification leads to an increase oxidized fibrinogen (i.e. its carbonyl derivative) level in the serum. This is evidenced by also our earlier study [15] on the influence of electromagnetic radiation 460 MHz on blood coagulation in rats, the results of which indicate that the fibrinogen is partially undergone to oxidative modification and don't form a fibrin clot. According to the work [16], fibrinogen, oxidized in vitro conditions, also disrupts coagulation process in plasma.

Antioxidant effect of irradiation at low-intensity with a power density of 10 mW/cm^2, which we believe is manifested

in a moderate reduction of protein carbonyl derivatives in plasma, may be associated with an increase in the total antioxidant activity of blood [authors' unpublished results]. In both cases, changes in lipid peroxidation rate detected in increasing or decreasing the concentration of MDA in blood consistent with changes in the oxidative modification of proteins.

Thus, depending of the intensity, decimeter microwave radiation can exert either pro- or antioxidant effect on such cell/tissue processes as lipid peroxidation and protein carbonylation. Both lipid peroxidation and protein carbonylation may mediate redox signaling processes, which may result in pathogenesis. Consequently, the development of methods using decimeter microwaves, which can control these processes in cells, should contribute to the development of the therapeutic strategies against various diseases.

References

[1] B. Brocklehurst, K. McLauchlan, "Free radical mechanism for the effects environmental electromagnetic fields on biological systems," Int. J. Radiat. Biol., v.69, N 1, pp.3-24, 1996.

[2] S.M. Zubkova, Biophysical and Physiological Mechanisms of Therapeutic Effects of Electromagnetic Radiation," Fizioterapiya, Balneologiya, Reabilitasiya, No 2, pp.3-9, 2002.

[3] A.M. Gadzhiev, E.Yu. Yusifov, M.T. Abbasova, L.F.Ismailova, A.B. Shabanova, Zh.M. İbragimova, P.A. Khalilov, N.R. Bagirova, "The study of microwave irradiation oxidative effect on living organism," Modern Problems of Comparative Physiology and Biochemistry, Baku, pp.278-290, 2005.

[4] A.V. Musaev, L.F. Ismailova, A.B. Shabanova, A.A. Magerramov, E.Yu. Yusifov, A.M. Gadzhiev, "Pro- and antioxidant effect of therapeutic microwave irradiation (460 MHz) on brain tissues in experiment," Voprosy Kurortologii, Fizioterapii, Lechebnoy Fizkultury, Moscow, No 2, pp.19-23, 2004.

[5] Y.J. Suzuki, M. Carini, and D.A.Butterfield "Protein Carbonylation," Antioxidants & Redox Signaling, Vol.12, Number 3, 2010.

[6] E.E. Dubinina, S.O. Burmistrov, D.A. Khodov, I.G. Porotov, "Oxidative modification of proteins in human blood serum, method for determining," Voprosy meditsinskoy khimii (Moscow), vol. 41, No 1, p.p.24-26, 1995.

[7] L.I. Andreeva, L.A. Kozhemyakin, A.A. Kishkun, "Modification of method for determining lipid peroxides in thiobarbituric asid test," Laboratornoe delo, pp. 41-43, 1987.

[8] R.L. Levine, D. Garland, C.N.Oliver, P. Amici J. Biol. Chem., 1987, vol. 262. pp. 9908-9913.

[9] A. Iehan, A. Gurel, F. Armutcu, Clin. Chim. Acta, 2004, vol. 340, No 1-2. p. 153

[10] F. Oktem, F. Orgunez, H. Mollaogly, Arch. Med. Res., 2005. vol.36. No4, pp. 350-355.

[11] E.E. Khirazova, A.A. Bayzhumanov, L.K. Trofimova, L.I. Deev, M.V. Maslova, N.A. Sokolova, N.Yu. Kudryashova, "Effects of electromagnetic radiation of GSM-band on some physiological and biochemical characteristics in rats," Bulleten experimental biology and medicine, 2012, vol. 153, No 6, pp. 791-794.

[12] A.V. Musaev, L.F. Ismailova, A.M .Gadzhiev, "Influence of SHF (460 MHz) electromagnetic fields on the level of induced lipid peroxidation in the structures of visual analyzer and hypothalamus in experimental animals," Voprosy Kurortologii, Fizioterapii, Lechebnoy Fizkultury, Moscow, No 5, pp.17-20, 2005.

[13] A.M. Gadzhiev, A.V. Musaev, L.F. Ismailova, "Effects of decimetric microwaves on thiol defense system in ocular structures of animals of different age," Fizioterapiya, Balneologiya and Reabilitasiya, Moscow, No 6, pp.13-17, 2005.

[14] I.V. Kaplan, M. Attaelmannan, S.S. Levinson, Atherosclerosis, 2001, vol. 158. pp.455-463.

[15] M.T. Abbasova, A.M.Hajiyev, "Influence of chronic electromagnetic irradiation on blood coagulation," Azerbaijan Medical Journal, 2010, No 3, pp. 9-13.

[16] O.A. Azizova, A.P. Piryazev, S.N. Moskvina, A.V.Aseychev, "The method for detection of protein oxidability in serum and plasma of blood," Biomeditsinskaya khimiya, Moscow, 2007, vol. 53, issue 1, pp. 99-106.

[17] A.V. Musaev, Zh.M. İbragimova, A.M. Gadzhiev, "Modification of experimental oxidative stress in lens tissues under effect of 460 MHz electromaqnetic radiation," Fizioterapiya, Balneologiya and Reabilitasiya, Moscow, No 2, pp.10-13, 2009.

[18] L.F. Ismailova, A.B. Shabanova, E.Yu. Yusifov, A.M. Gadzhiev, "Adaptive changes of redox status in rat brain tissues due to decimeter microwave irradiation," Iranian Journal of Pharmaceutical Research, 2004, vol. 3, pp. 19. Iranian Neuroscience Congress May 16-19, Tehran, Iran.

[19] A.M. Gadzhiev, L.F. Ismailova and A.B. Shabanova, "Adaptive changes of redox status in rat brain tissues due to decimeter microwave irradiation," Journal of Neurochemistry, 2004, vol. 88. pp. 9-1, Hong Kong, 4-7 February.