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Haptoglobin and Lipid Peroxidation Induced by Electromagnetic Radiation

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Abstract - The participation of free radical oxidation processes is suggested to occur in the mechanism of biological effects of electromagnetic radiation (EMR) that finds more evidence in experimental facts. For inhibition of free radical reactions, including process of lipid peroxidation, there is an antioxidant system in tissues and organs. Haptoglobin as an antioxidant involved in the regulation of hormonal processes and lipid peroxidation. The aim of present work was to study the effect of whole body irradiation with decimeter electromagnetic radiation (460 MHz) on blood haptoglobin level. Results show that prolonged exposure of animals up to 3 weeks at power density of 30 mkW/cm^2 leads to increased serum haptoglobin level (34.72±1.87 mg%), whereas control animals' level of haptoglobin was at 24,05 ± 0.73 mg % ($p<0,05$). In our experiments, we also have shown that EMR 460 MHz for a prolonged irradiation under the same conditions leads to increased concentrations of lipid peroxidation products in blood of rats. Increasing of haptoglobin concentration in blood is probably a compensatory effect to prevent oxidative damage of the cellular elements.

Keywords - *electromagnetic radiation, haptoglobin, lipid peroxidation, plasma, erythrocytes*

I. INTRODUCTION

One of the important processes related to the cell and the cell membrane is lipid peroxidation (LPO). The regulation of this process is realized by specific cellular antioxidant substances. Haptoglobin as an antioxidant involved in the regulation of hormonal and peroxidation processes. One of haptoglobin functions is the binding of hemoglobin released from disrupted red blood cells, and thus preventing the loss of iron by organism. Furthermore, haptoglobin participates in compensatory reactions of organism, affects the immune status, performs a protective function. Also, its complex associated with hemoglobin acts as a peroxidase - an enzyme that promotes the oxidation of various organic substances by peroxides [1, 2].

Non-ionizing electromagnetic radiation are widely used in people's life. In recent decades, one might say, a mobile radiation affects entire population. Biological consequences of these actions and implementation mechanisms are practically unknown and little studied to date. However, there are assumptions about the nature of free radical action of such low-energy radiation on living tissue, which find some indirect evidences in few experimental works. In particular, in our earlier studies,we have shown the influence of UHF radiation on process of lipid peroxidation in different organs in totally irradiated rats [3, 4]. There are also some other work in this direction of investigation [5-7]. In this study, we attempted to obtain new data on the redox character of the action of ultrahigh frequency electromagnetic radiation on the blood of animals in their whole-body irradiation.

Thus, the aim of our experimental study was to investigate the changes in the plasma haptoglobin level in organism chronically exposed to whole body non-ionizing irradiation parallel to the measurements of intensity of lipid peroxidation in erythrocytes.

II. MATERIAL AND METHODS

Investigations were carried out on Wistar albino rats weighting 250-300 g were kept under normal conditions of the vivarium. Animals were randomly divided into experimental and control groups. The experimental group of animals irradiated with decimeter radiation (460 MHz) using the "Volna-2" (made in Russia) in a metal cylindrical chamber. Irradiation was carried out daily for 20 minutes to one month at a flux density of 30 mkW/cm^2. The content of malon dialdehide (MDA) in the red blood cells was determined by the method proposed by Suplotov and Barkova (1986) [8]. A haptoglobin content was determined by the method Prohurovskaya and Movshovich (1972) [9]. The principle of the method is as follows: after addition to serum a fixed amount of hemoglobin, hemoglobin-haptoglobin complex formed in serum precipitates by rivanolum. For determining of haptoglobin content, 3 samples were measured: serum with addition of Hb solution where Hp-Hb complex was formed (experience), serum without adding Hb (control) and Hb solution in distilled water (standard).
The tube was poured with vigorous stirring 0.5 ml unhemolyzed serum, 0.2 ml of 0.5 g% Hb solution and 0.3 ml of distilled water. After 10 minutes, 2 ml of 0.3% rivanolum was added at stirring. After another 5 minutes, the mixture was centrifuged at 3000 rev/min. The supernatant was decanted into a clean tube. To complete bleaching was added 0.2 ml of 10% solution of $(NH_4)_2SO_4$. After standing at room temperature for one hour, photometric measurements carried out on the FEC-M with a green filter against water in a cuvette with a 1 cm.

Control. Progress of the reaction is the same as in the experiment. To 0.2 ml of serum, instead of 0.5 g%

Hb solution was added the same amount of distilled water.

Standard. To 2.8 ml of distilled water is added 0.2 ml of 0.5 g% Hb solution and 0.2 ml of a 10% solution of $(NH_4)_2SO_4$.

Calculation. In Hb-Hp complex, because of two substances contained in equal weight ratios, calculation is performed according to the formula:

$$Hp\ (in\ mg\%) = 200 \bullet (E_s - E_e + E_k)/E_s$$

where E_s - optical standard density; E_e - optical density of experiment; E_k - optical density of control. In those cases where the number of experiences is greater than 200 mg Hp%, measurements are carried out repeatedly by adding double amount of Hb to serum, and a coefficient 200 in the formula was changed to 400.

To determine the content of MDA in the red blood cells, 0.1 ml of washed erythrocytes haemolysed with 2.0 ml distilled water. Thereafter, 1.0 ml of 17% trichloroacetic acid and 1.0 ml of 0.8% 2-thiobarbituric acid were added to hemolysate and heated on a boiling water bath for 10 min, centrifuged 10 min at 3000 rev/min. To control sample instead of red blood cells was added 0.1 ml of distilled water. The optical density of colored product was measured at a wavelength of 540 nm on spectrophotometer SF using 1 cm thick cell against a control sample. The calculation was carried out based on the molar extinction coefficient of malondialdehide $1.56 \cdot 10^5$ cm^{-1} M according to the formula:

$$A = \frac{E_o \cdot 10^5}{1{,}56 \cdot 10^5 \cdot 0{,}1ml} = E_o \cdot 64{,}1$$

where A - the content of MDA nmol/ml, E_0 - absorbance of test sample, 0.1 ml - volume of red blood cells.

III. RESULTS AND DISCUSSION

Studies have revealed that under the influence of electromagnetic radiation of ultrahigh frequency, the intensity of lipid peroxidation increased and this depends on the total time of exposure to EMR. Exposure 460 MHz EMR at irradiation intensity with power flux density of 30 mkW/cm^2 for 1 month resulted in a significant accumulation of lipid peroxidation product malondialdehyde in rat erythrocytes. The content of erythrocyte MDA for the control and irradiated rats showed the following values: control – 12.44±0.14 nmol/ml; irradiated – 15.83±1.52 (7 days); 17.86±1.9 (14 days); 13.70±0.09 (21 days); 16.07±0.30 nmol/ml (28 day) (Fig.1).

Changes in the level of haptoglobin in the blood of rats exposed to EMR 460 MHz at a power flux density of 30 mkW/cm^2 are shown in Fig.2. As shown, the haptoglobin level for irradiated rats - 33.82±2.32 (7 days); 35.8±1.5(14 days); 34.65±1.8 (21 days); 23.8±3.0 mg% (28 days) consistently higher than that of control rats (24.05±0.73 mg/%). The significance of difference was of $p<0.05$ and $p<0.01$.

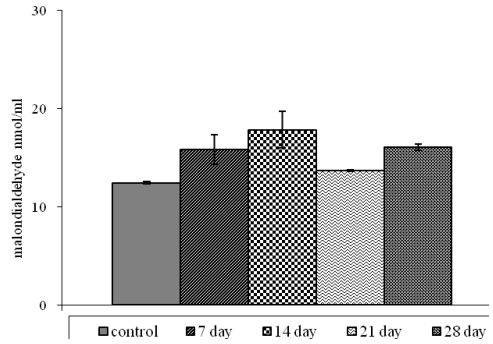

*Fig.1. Changes of lipid peroxidation product malondialdehyde content in erythrocytes of rats exposed to 460 MHz radiation during 1 month, 20 min a day. Power density was 30 mkW/cm^2. Mean ± SE (n = 5). * - p<0.05; ** - P <0.01 irradiated group versus control one.*

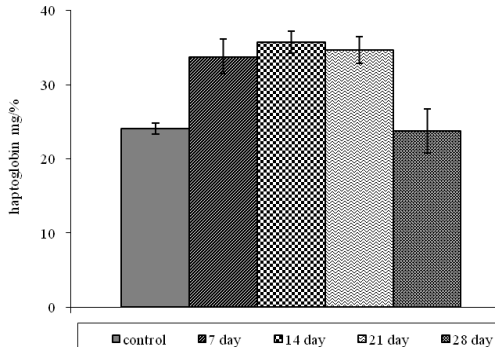

*Fig.2. Plasma haptoglobin content in rats exposed to radiation 460 MHz within 1 month, 20 min a day. Power density was 30 mkW/cm^2. Mean ± SE (n=5). * - p<0.05; ** - p <0.01 irradiated group versus control one.*

Thus, we see that the content of lipid peroxidation products in the blood of the animals increases under the influence of EMR exposure of 460 MHz, that indicates the strengthening of free radical processes in organism. On the other hand, significance increase of haptoglobin concentration in blood is observed under the same conditions of irradiation of animals that is apparently associated with increased oxidative stress in tissues. Increasing of haptoglobin concentration is a compensatory effect to prevent damage of the cellular elements.

It is known that various oxidative effects on red blood cells lead to oxidation and denaturation of the hemoglobin which accompanied by the release of heme / hemin [10]. It is well known that the exogenous hemin easily integrates into the membrane, destabilizing it and causing hemolysis. Erythrocyte dehydration induced by oxidation is the result of increasing in concentration of intracellular Ca^{2+} and activation of Gardos channels

[11, 12]. And there is evidence that lipid peroxidation products activates phospholipase, which initiates cyclo- and lipoxygenase reactions of conversion of membrane phospholipids into cyclic nucleotides. Amplification peroxidation in the cell membrane alters the conductivity of calcium channels and decreases calcium-binding ability of cell membrane phospholipase [13].

The main physiological function of haptoglobin is to keep the iron in organism. In addition, the hemoglobin-haptoglobin complex has a high peroxidase activity, which has an inhibitory effect on lipid peroxidation.

Thus, relatively high frequency microwaves cause significant disturbances in oxidative processes in blood/organism, which speaks in favor of free radical mechanism of biological action of electromagnetic radiation.

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