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THE EFFECT OF 50 Hz MAGNETIC FIELD OF DIFFERENT SHAPE ON OXYGEN METABOLISM IN BLOOD PLATELETS: *IN VITRO* STUDIES

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Abstract
Objectives: The aim of the study was to assess the influence that the shape of low frequency magnetic field may have on catalase and superoxide dismutase activity, malondialdehyde concentration and free radicals generation in human blood platelets. **Materials and methods:** The suspension of human blood platelets was exposed for 15 min to 50 Hz magnetic field of different shape, and flux density of 10 mT. **Results:** The determinations of free radicals, malondialdehyde and catalase showed increased values compared with the initial level, regardless of the shape of the magnetic field applied. In contrast, superoxide dismutase activity was lower than at the onset of the experiment. **Conclusions:** The findings indicate that the oxidative stress resulting from exposure to 50 Hz magnetic field of 10 mT induction may produce a number of adverse effects within the cell and thus may lead to systemic disturbances in the human body.

Key words:
Electromagnetic Field, Blood Platelets, Oxygen Metabolism

INTRODUCTION

Electromagnetic field (EMF) is an essential element of the natural environment. It helps sustain the life processes of the plants, animals, and humans as well as their biological and physiological functions. Throughout the evolution processes, the living organisms had developed numerous adaptation mechanisms to natural electromagnetic fields that enabled them to survive under the changing environmental conditions. However, the progress of civilization and the technological development has brought about the invention of emitters of artificial electromagnetic radiation. The abundance and variety of the sources of artificial EMF gives rise to the so-called electromagnetic smog. Long-term exposure to this factor may produce a number of biological effects, these being dependent both on the radiation parameters and the properties of biological structures. Artificial electromagnetic fields (magnetic fields in particular) were found to have influence on the systemic integrative and regulatory functions. Thus long-term EMF exposure may change the tolerance limit and lead to the manifestation of various dysfunctions, including subjective and objective functional changes. Depending on the frequency, the variable magnetic field may exert either positive or negative biological effects.

Data from the Polish and foreign literature show the range of frequency and magnetic induction of the stimulus used for the therapeutic purposes. There are also numerous

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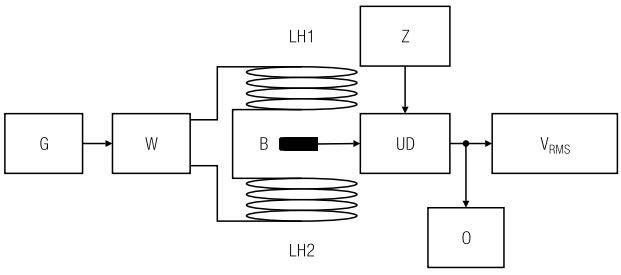
reports on the adverse effects of radiation on the level of reactive oxygen species (ROS) and the changes in enzymatic activity and antioxidant defence mechanism in experimental models. In these studies, different ranges of frequency and magnetic flux density were examined with respect to their effect on oxidative stress in the exposed cells. However, there are no studies that would compare the effects of electromagnetic radiation taking into account the different shapes of EM field. Therefore, the present study was undertaken to assess the effect of the shape of low frequency magnetic field (MF) on the activity of superoxide dismutase (Cu, Zn–SOD) and catalase (CAT), and to determine the level of free radicals and malondialdehyde concentration (MDA-TBARS) in human blood platelets. The studies were conducted *in vitro.*

EXPERIMENTAL PROCEDURES

The suspension of human blood platelets at the concentration of $1x10^9/cm^3$, obtained from voluntary blood donors at a blood donation centre, was the material for the study. The suspension was obtained from whole blood by manual apheresis. The donors underwent medical examinations (those who had contraindications for blood drawing were excluded) and laboratory tests typical for blood donors were performed. Blood platelet suspension was exposed to MF of different shape, 50 Hz frequency and 10 mT flux density (rms value) for 15 min. Helmholtz coils were used, of the mean radius of 60 mm and rectangular cross-section of 20×13 mm. The coils were positioned with 40-mm spacing on a support column into which the test tubes with blood platelet suspension were inserted. Then, they were exposed to MF of specific parameters. The coils generated electromagnetic field acting on blood platelets placed in six polyethylene tubes, each containing maximum 3 cm^3 of blood preparation. Helmholtz coil geometry and spacing were selected in such a way that the magnetic component of the field stimulating the blood platelets had a uniform course and induction of the values predefined before the study. The volume of the uniform field had the form of a cylinder that was in a coaxial and symmetric relationship with the coils. The cylinder height of 32 mm radius was equal to the spacing between the coils decreased by 10 mm, that is 30 mm.

Figure 1 presents a block diagram of the electrical system used in the study. The time course of MF found in the space between Helmholtz coils connected in series (LH1, LH2) determines the course of the output signal of generator G. In the system, HM8150 generator (HAMEG) was used as the source of sinusoidal, triangular or rectangular signal of regulated rms value and 50 Hz frequency. Amplifier W (W-320) adjusts small impedance (20 ohms) of Helmholtz coils connected in series to high output impedance (600 ohms) of generator G. Sensor B was used to observe the time course and to measure the rms value of magnetic component of the field stimulating the platelets tested. Polyethylene tube with magnetic field transducer SS49 (Honeywell) installed inside and connected to a digital multimeter 3860M (RIGOL) was used as the sensor. During the testing, the tube can be placed in one of the eight holes (Fig. 1) of the stand for placing the tubes with platelets. The set for measuring the induction of magnetic component of EMF between Helmholtz coils was calibrated with teslometer TH26. The teslometer probe and the sensor were placed in the adjacent holes of the laboratory stand.

The oxidative stress parameters were measured before and immediately after the exposure. The level of free radicals, and activity of superoxide dismutase and catalase as a part of the antioxidant defence system as well as malondialdehyde concentration as a marker of cell membrane



LH1, LH2 — Helmholtz coils, B — magnetic field sensor, G — signal generator, W — measuring amplifier, O — oscilloscope, Z — power supply 5V DC, UD — electronic signal conditioner, V_{RMS} — true RMS voltmeter.

Fig. 1. Block diagram of the electrical system applied in the experiment.

peroxidation were determined. The obtained results were subjected to statistical analysis.

Free radicals generation in blood platelets was determined by chemiluminescence using Lumicom luminometer (HAMILTON) connected to IBM PC. Simultaneous sequential measurement was performed for six samples from the control and study group. The control sample consisted of platelet suspension with PBS and luminol, whereas the study sample of EMF-stimulated platelet suspension with PBS and luminol. The test was performed at 25°C for 30 min.

To determine superoxide dismutase activity in blood platelets, 0.8 cm^3 of H_2O redestilled and cooled to +4°C and 0.5 cm^3 of 96% C_2H_5OH and 0.25 cm^3 of chloroform were added to the suspension at the concentration of $1\times10^9/cm^3$. The obtained mixture was shaken for two minutes and then centrifuged at 4200×g at +4°C for 10 min. After centrifugation, the enzyme remained in the upper layer of the suspension. Then 0.2 cm^3 of supernatant was transferred into glass tubes. The blind test contained 2.8 m^3 0.25 M carbonate buffer of pH = 10.2 and 0.2 cm^3 of adrenaline solution. In contrast, the study sample included 2.6 cm^3 0.5 M carbonate buffer of pH = 10.2 plus 0.2 cm^3 supernatant plus 0.2 cm^3 adrenaline solution. Spectrophotometer CARY 100 B10 (VARIAN) was used for the measurements and the determinations were performed at 480 nm wavelength. Absorbance in the control and study samples was measured every 1 min at +25°C for 10 min. Superoxide dismutase activity was calculated on the basis of absorbance reading from the analytical curve, or from appropriate mathematical formula. The values were presented in U/g of platelet protein. The amount of enzyme which causes a 50% inhibition at the maximal increase of absorbance by 0.025 of unit per minute on a rectilinear segment of adrenochrome formation at +25°C at 480 nm, is defined as a unit of enzymatic activity of superoxide dismutase [1].

To determine the catalase activity, 1 cm^3 of blood platelet suspension at the concentration of $1\times10^9/cm^3$ was used. It was frozen and thawed several times before the cell disintegration occurred. Then the whole system was centrifuged at 4300×g at +4°C for 10 min, and a clear supernatant was obtained. The control samples contained 3 cm^3 0.05 M phosphatic buffer, whereas the study samples — 2 cm^3 0.05 M phosphatic buffer plus 50 µl supernatant plus 1 cm^3 H_2O_2. Catalase activity was determined on the same spectrophotometer as above at 240 nm wavelength and at +25°C, and absorbance was measured every 1 min for 5 min. Catalase activity was calculated on the basis of absorbance reading from the analytical curve, or from appropriate mathematical formula. The obtained values were presented in Bergmeyer units per gram of platelet protein [2].

To determine malondialdehyde concentration, equal volume of 20% triochloracetic acid (TCA) was added to blood platelet suspension at the concentration of $1\times10^9/cm^3$. The mixture was shaken for 1 h at +4°C and then centrifuged at 4200×g at +4°C for 15 min. To 1.8 cm^3 of the obtained supernatant, 0.4 cm^3 0.12 M thiobarbituric acid was added. The mixture was placed in boiling water bath for 15 min. After cooling, the obtained solution was centrifuged at 3000×g for 10 min at room temperature. The absorbance was measured at 532 nm wavelength. The results are expressed as nmol/10^9 of platelets [3].

Basic parameters were used in the description of the study variables: arithmetic mean (x), standard deviation (SD) and median (m_e). Median is the most important parameter due to the distribution of the study variables (non-normal distribution). The obtained results were analyzed using a nonparametric Kruskal-Wallis Anova rank test (equivalent to analysis of variance), as well as Mann-Whitney test to compare the variables between the groups and Shapiro-Wilk test (analysis of variable distribution). The value of $p < 0.05$ was considered the level of confidence.

RESULTS

Oxygen activity in blood platelets, expressed by the generation of free radicals stimulated by 10 mT magnetic induction and 50 Hz frequency, increased significantly after 15-min exposure, compared to the control values (Fig. 2). The shape of the applied MF was monitored to determine the number of free radicals generated in blood platelets. Statistically significant differences were

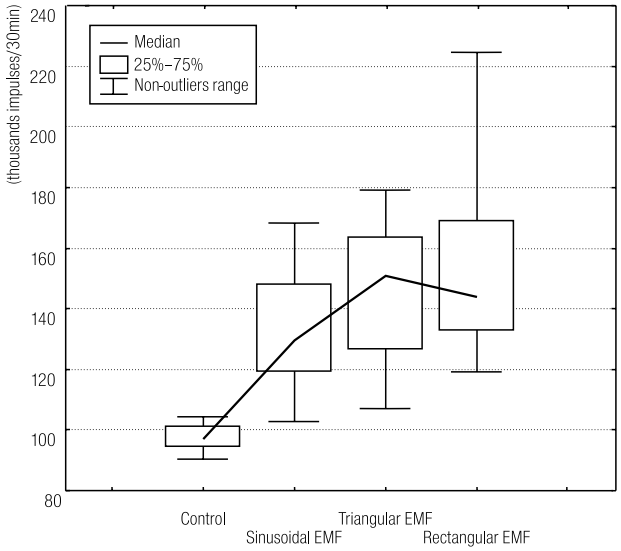


Fig. 2. Determination of free radicals (luminescence method) in EMF-exposed blood platelets in relation to MF shape.

found between the level of free radicals in blood platelets exposed to sinusoidal MF (x = 133.10 impulses/30 min) for 15 min and the triangular MF (x = 154.26 impulses/30 min) and between the sinusoidal MF (x = 133.10 impulses/30 min) and rectangular MF (x = 147.28 impulses/30 min). However, no such differences were detected in comparison between the triangular and rectangular shape of the field. A highest increase in relation to the initial values (by 53.4%) of the median of free radicals level was noted after application of the triangular impulse.

The activity of superoxide dismutase (Cu, Zn-SOD) in blood platelets exposed to MF for 15 min decreased significantly compared to the control values, and the enzymatic activity depended on the shape of MF applied (Fig. 3). The highest decrease, compared to the initial values, was observed when blood platelets were exposed to MF of triangular and rectangular shape. After 15 min of EMF radiation of rectangular shape, the value of x = 2139.07 U/g decreased to x = 876.19 U/g (enzymatic activity decreased by 59%). When the triangular field was applied, the median of superoxide dismutase activity decreased by 54% of the initial value (to x = 981.87 U/g).

After 15 min exposure of blood platelets to 10 mT magnetic flux density and 50 Hz frequency, catalase activity (CAT) increased significantly compared to the control level and the obtained values depended on the shape of MF applied. The highest increase, by 42.5% (x = 6.49 U/g to x = 9.25 U/g), was noted after application of the triangular field, whereas the lowest one, by 8.9% (x = 6.49 U/g to x = 7.07 U/g), when the sinusoidal MF was applied. A statistically significant dependence was found between the effect of the triangular and rectangular MF. No such relationship was observed for the effect of the sinusoidal and rectangular or sinusoidal and triangular MF (Fig. 4).

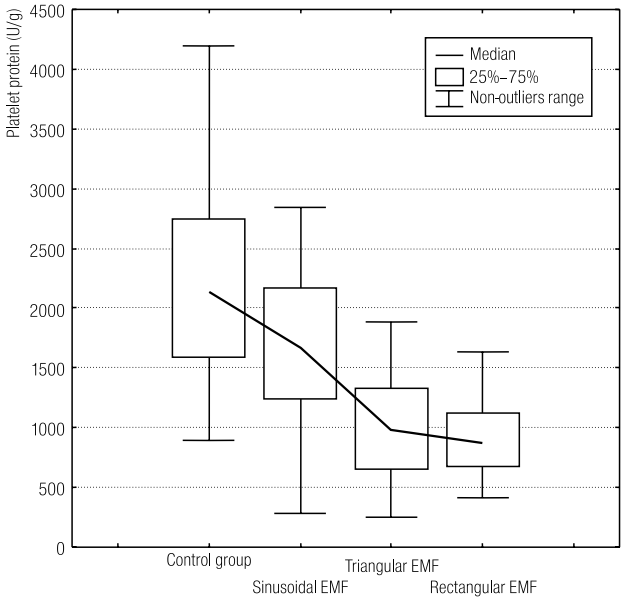


Fig. 3. Superoxide dismutase (SOD-1) activity in EMF-exposed blood platelets in relation to MF shape.

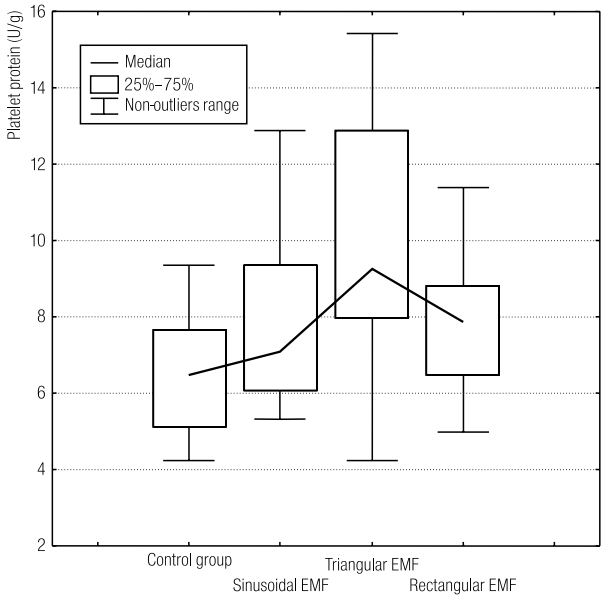


Fig. 4. Catalase (CAT) activity in EMF-exposed blood platelets in relation to MF shape.

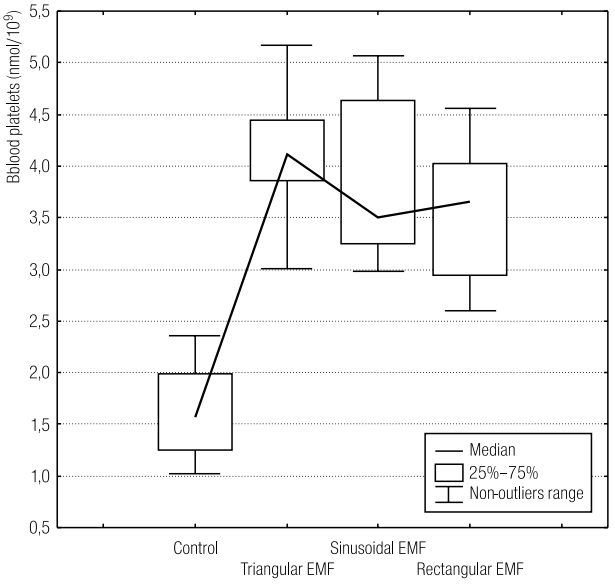


Fig. 5. Malondialdehyde (MDA) concentration in TBARS of EMF-exposed blood platelets in relation to MF shape.

After 15-min exposure to MF, the malondialdehyde (MDA) concentration in blood platelets increased in relation to the initial level. The concentration depended on the shape of the MF applied (Fig. 5). The highest increase was noted after exposure to MF of the sinusoid pulse oscillation ($x = 1.57$ nmol/10^9 to $x = 4.12$ nmol/10^9). Moreover, significant differences were found between MDA concentrations when electromagnetic field of the sinusoid shape was applied ($x = 4.12$ nmol/10^9), compared to the rectangular shape ($x = 3.65$ nmol/10^9).

DISCUSSION

The first reports on the adverse effects of electromagnetic field on humans were published at the end of 1970s. The field generated by electric power transmission line induced an increase in the prevalence of leukaemia and cerebral tumours in children living in the vicinity of the power line [4]. Moreover, increased mortality from all kinds of neoplasms and leukemias was observed in some age groups, particularly among younger subjects (0–14 years) living in the vicinity of AM radio broadcasting towers [5]. In other reports, the authors point to the lack of association between environmental exposure to this factor and the prevalence of leukaemia, cerebral tumours or breast cancer [6]. Owing to numerous, often controversial, reports on the potentially carcinogenic effect of EMF exposure, the scientists are inclined to agree that the electromagnetic field is the so-called epigenetic factor. It means that EMF enables or accelerates the growth of a neoplasm induced by another factor but it does not initiate the disease [9].

Scientific reports provide information on the cardiovascular effects of EMF exposure. Disorders were found in the regulation of arterial blood pressure and heart rate in persons working in outdoor switch-yards [8]. Another study demonstrated an increased risk of death due to myocardial infarction and arrhythmia among electric utility workers excessively exposed to EM radiation [9].

There are also studies which suggest that occupational exposure to low frequency EMF may result in disturbances of the neurovegetative regulation of the cardiovascular function [10].

To induce the systemic effects, the electromagnetic field must exert influence on the intracellular mechanisms. The changes taking place on the molecular level account for the response of the organism as a whole. The main task for researchers is to determine the ranges of frequency and magnetic induction of EMF that may cause adverse health effects or be safe to exposed humans [11].

Analyzing the effects that EMF may have on the biological systems, one should pay attention to numerous studies reporting an increase in free radicals generation in these systems. Uncontrolled increase in the level of reactive oxygen species can lead to the oxidation of important cell structures: proteins, lipids, saccharides, or even to the damage of the genetic material [12]. The consequences of oxidative stress can be severe — they can lead to an increased number of mutations or to cell death [13]. Scientific reports also point to the changes in the antioxidant defence systems, both with respect to the formation of low-molecular antioxidants and enzymatic systems. These data indicate the direction of the present and future research. The efforts of the researchers are focused on determining the molecular effects of EMF exposure.

Cell oxygen metabolism, this referring also to blood platelets, is the so-called biological window for EM radiation.

Biological windows are the structures or functions that undergo changes resulting from increased susceptibility to exogenous EM radiation. Electromagnetic field with defined parameters is an environmental factor which can cause increased generation of free radicals and other ROS in the cell. Free radicals are defined as atoms or molecules capable of independent existence and having one or more unpaired electrons in the valence shell. Free radicals are usually characterized by high reactivity, which means that they readily bind to different molecules.

The extent of damage caused by free radicals depends on the balance between the rate of their generation and the concentration of low-molecular antioxidants and the activity of antioxidant defence enzymes. The conditions under which this balance becomes impaired, leading to an increase in the stationary concentration of free radicals, is defined as oxidative stress.

Own studies investigating the effect of the shape of MF of 10 mT flux density and 50 Hz frequency showed an increase in the level of ROS, compared to the control samples, after blood platelet exposure to EMF radiation. Statistically significant differences were noted after 15-min exposure regardless of the shape of the impulse. The most significant changes were detected when EMF of the triangular and rectangular pulse of oscillation was applied. The increase in ROS level in the study samples may indicate a delicate biological balance between their generation and elimination from the environment.

Similar findings were reported in the interesting studies on the effect of 1000 Hz MF with 0.5 mT flux density on oxygen metabolism in blood platelets. The field parameters were those found in motor vehicles. An increase in ROS generation was found after 30-min and 90-min exposure. However, after 60-min exposure, the number of free radicals decreased significantly and remained at the level found in the control samples, which indicates that a biological balance has been reached between their generation and elimination [14].

Other authors also emphasize the effect of EMF radiation on free radicals in the exposed biological structures. Zmyślony et al. demonstrated that ROS generation in rat lymphocytes increased after exposure to 930-MHz EM radiation [15].

In our study, the level of free radicals, superoxide dismutase and catalase activity, and malondialdehyde concentration in blood platelets point to the significance of the shape of MF field that the biological systems are exposed to. At present, there is no experimental data that would explain this phenomenon. Cells are protected against adverse effects of MF exposure, among others, via enzymatic proteins that form a part of the antioxidant defense system. Catalase is one of such proteins. It consists of four subunits and can be found in eucariotic cells in two forms: as cytoplasmic and peroxisomal catalase. Catalases convert the disproportionate hydrogen peroxide to water and molecular oxygen. This reaction is of importance because hydrogen peroxide is a substrate of the Fenton's reaction which leads to the generation of the most reactive oxygen form, namely hydroxyl radical.

In the studies investigating the effect of exposure to 1000 Hz and 0.5 mT MF radiation on the activity of enzymes of the antioxidant defense system in blood platelets, a significant increase in catalase activity was observed after 30-min exposure, which correlated with an increase in ROS generation. However, further measurements, after 60 and 90 min of exposure, demonstrated a decreased activity of this enzyme, compared to the values found for the control samples [14].

Under conditions of 8-min exposure to MF radiation of 70 μT flux density, the authors of another study also detected a decrease in catalase activity, which correlated with a significant decrease in H_2O_2 concentration. A significant decrease in activity referred to the enzymes from the group of metalloenzymes [2].

The findings of own studies demonstrated an increase in catalase activity in blood platelets regardless of the shape of magnetic impulse after 15-min exposure to 50 Hz and 10 mT EM radiation. The highest increase, compared to the control samples, was observed in exposure to EMF with triangular pulse oscillation. This increase can be explained by cellular defence against the excess of hydrogen peroxide.

An uncontrolled increase in the concentration of free radicals leads, among others, to lipid peroxidation. This process consists in oxidation of polyunsaturated fatty acids

contained in phospholipids which are the main component of biological membranes. Lipid peroxidation affects cell membrane fluidity which in turn increases membrane permeability and depolarisation. Furthermore, it is postulated that free radicals may have an effect on the function of membrane channels: potassium, sodium and calcium [16]. The compounds reacting with thiobarbituric acid (TBARS), including malondialdehyde (MDA), are the end-products of lipid peroxidation. MDA determination is a marker of this process.

Aldehydes, including malondialdehyde, are less reactive than free radicals. Thus, they can diffuse to distant cellular structures where they can cause further damage, including DNA damage. Owing to these properties, they are said to have citotoxic, mutagenic and carcinogenic potential [7].

Lipid peroxidation, the course of which is rapid and leads to oxidation of unsaturated fatty acids by ROS, which in turn results in the generation of lipid peroxides, is an important process that needs consideration. Cell membranes are a frequent site of attack because they contain phosholipids which are built of polyunsaturated fatty acids. Thus, lipid peroxidation produces numerous consequences including changes in the structure and fluidity of cell membrane, disturbances in membrane transport, changes in the activity of cell membrane enzymes or damage of protein receptors located in membrane structures [17].

Increased lipid peroxidation was observed for steady MF of flux density of ca. 8 mT [18]. Artificial phospholipid membranes were used in this test. Zmyślony et al. demonstrated an increase in lipid peroxidation in rat liver microsomes as a result of exposure to 5 mT EM radiation [17]. Other researchers also reported increased level of lipid peroxidation in brain homogenates of mice exposed to MF of 50 Hz frequency and 1 and 5 mT induction for 30 days for 3 h/daily [19]. Furthermore, also exposure to EMF of power frequency and 1 and 5 mT induction induced oxidative stress in brain cells of rats [20].

In the studies on the effect of car electronics on oxygen metabolism in blood platelets, a statistically significant increase in malondialdehyde activity was detected after 30-, 60- and 90-min exposure to EM radiation [21]. Also the electromagnetic radiation emitted by mobile phones is of significance for lipid peroxidation. Significant increase in MDA activity, compared to the values found for control samples, was detected in blood platelets exposed to 900 MHz microwave radiation for 1, 5 and 7 min. This was due to the extrathermal effect [22].

Similar results were obtained in the present study. The level of MDA was used as a marker of lipid peroxidation. MDA is one of the end-products of lipid peroxidation and belongs to the compounds reacting with thiobarbituric acid. The increase in MDA activity coupled with increased free radicals generation points to the damage of cell membranes by free radicals. After a 15-min exposure of blood platelets to MF radiation, the MDA level increased significantly, compared to the control value. The highest increase could be noted after a short-term exposure to MF of sinusoid pulse oscillation, which indicates an adverse effect of electromagnetic radiation within the range of the study parameters on oxygen metabolism in blood platelets.

CONCLUSIONS

1. The increase in the number of free radicals after exposure to low frequency electromagnetic field may point to adverse effects on oxygen metabolism in blood platelets and to their high sensitivity to this environmental factor.
2. The decrease in the antioxidant activity of superoxide dismutase and the increase in catalase activity may be the cause of adverse effects of MF exposure on oxygen metabolism in blood platelets.
3. The findings of the present study indicate that the oxidative stress which develops during MF exposure may lead to lipid peroxidation in the cell membrane of blood platelets, which is expressed by a significant increase in malondialdehyde concentration.
4. The level of free radicals, superoxide dismutase and catalase activity and malondialdehyde concentration in blood platelets point to the significance of the shape of MF that the biological systems are exposed to.

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