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## The Impact of Electromagnetic Radiation of Different Parameters on Platelet Oxygen Metabolism – *In Vitro* Studies

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;  
D – writing the article; E – critical revision of the article; F – final approval of article; G – other

### Abstract

**Background.** Electromagnetic radiation emitted by a variety of devices, e.g. cell phones, computers and micro-waves, interacts with the human body in many ways. Research studies carried out in the last few decades have not yet resolved the issue of the effect of this factor on the human body and many questions are left without an unequivocal answer. Various biological and health-related effects have not been fully recognized. Thus further studies in this area are justified.

**Objectives.** A comparison of changes within catalase enzymatic activity and malondialdehyde concentration arising under the influence of the electromagnetic radiation emitted by car electronics, equipment used in physiotherapy and LCD monitors.

**Material and Methods.** The suspension of human blood platelets at a concentration of  $1 \times 10^9/0.001 \text{ dm}^3$ , obtained from whole blood by manual apheresis, was the study material. Blood platelets were exposed to an electromagnetic field for 30 min in a laboratory stand designed for the reconstruction of the electromagnetic radiation generated by car electronics, physiotherapy equipment and LCD monitors. The changes in catalase activity and malondialdehyde concentration were investigated after the exposure and compared to the control values (unexposed material).

**Results.** An increase in catalase activity and malondialdehyde concentration was observed after 30 min exposure of platelets to EMF regardless of the radiation source. The most significant changes determining the degree of oxidative stress were observed after exposure to the EMF generated by car electronics.

**Conclusions.** The low frequency electromagnetic fields generated by car electronics, physiotherapy equipment and LCD monitors may be a cause of oxidative stress in the human body and may lead to free radical diseases (**Adv Clin Exp Med 2015, 24, 1, 31–35**).

**Key words:** electromagnetic radiation, oxygen metabolism, catalase, malondialdehyde.

In recent decades, together with the increasing development of electrical devices, there appeared in human living and working environments numerous emitters of artificial electromagnetic radiation (EMF). This has resulted in an increase of the mean intensity of the electromagnetic field, which has given rise to so-called electromagnetic smog.

Electromagnetic waves of different frequency and intensity can create various threats to human health. Different organs and cell structures can be the site of electromagnetic radiation exposure in the body. Despite numerous publications [1, 4,

8–13] related to this subject, no unequivocal conclusions have been drawn which would point to specific dysfunctions and pathologies induced by the given parameters of electromagnetic radiation. Because of that, the authors undertook research on the impact of the EMF emitted by car electronics, physiotherapy equipment and display screens on the selected parameters of platelet oxidative stress: the changes in enzymatic activity of the antioxidant defense protein catalase and in the concentration of malondialdehyde, a marker of lipid membrane peroxidation.

## Objectives

The aim of the study was the comparison of changes in selected parameters of oxidative stress (enzymatic activity of catalase, malondialdehyde concentration) occurring under the influence of the electromagnetic radiation emitted by car electronics, physiotherapy equipment and LCD monitors.

## Material and Methods

The suspension of human blood platelets at a concentration of  $1 \times 10^9/0.001 \text{ dm}^3$ , obtained from whole blood by manual apheresis, was the study material. The preparation was obtained from the Blood Donation Center from blood donors who underwent medical examinations, and laboratory tests of the blood were performed.

To expose the suspension to radiation, a special device was built in laboratory conditions, reconstructing the parameters of the electromagnetic field generated by car electronics (1 kHz, 0.5 mT), physiotherapy equipment (50 Hz, 10 mT) and LCD monitors (1 kHz, 220 V/m). When electromagnetic radiation of a low frequency is tested, the electrical and magnetic components should be investigated independently. In the presented studies on the sources of the electromagnetic field of LCD monitors, electric component was considered, as its parameters appeared to be of significance as regards their effect on the human body. The electromagnetic field emitted by car electronics and physiotherapy equipment was characterized by the magnetic component. In the case of LCD monitors, a flat capacitor formed by 2 circular copper plates was the source of the electromagnetic field. The plates were positioned over and under a plastic support in which 8 polyethylene tubes containing the tested suspension were inserted into holes. The volume of the tested preparation did not exceed 3 mm and the preparation was exposed to the EMF for 30 min. To recreate the radiation emitted by car electronics and physiotherapy equipment, Helmholtz coils were used. Their geometry and spacing were selected in such a way that the magnetic component of the field stimulating the preparation had a uniform course and its induction was characterized by the values occurring in vehicles or the equipment used in physiotherapy.

The selected parameters of oxidative stress were tested before and immediately after the exposure. The enzymatic activity of catalase and malondialdehyde concentration (MDA), as lipid peroxidation marker, were determined.

To determine the catalase enzymatic activity, the suspension of blood platelets was frozen and

thawed several times before cell disintegration occurred. After centrifugation of the whole system at  $4200 \times g$  at  $+4^\circ\text{C}$  for 10 min, a clear supernatant was obtained. The measurement was performed using a CARY 100 BIO (VARIAN) spectrophotometer at 240 wavelength at  $+25^\circ\text{C}$  and absorbance was measured every minute for 5 min. Thirty six control and exposed samples were used. The control sample contained  $0.003 \text{ dm}^3$  0.05 M phosphate buffer, whereas the study sample had  $0.002 \text{ dm}^3$  0.05 phosphate buffer, 50  $\mu\text{L}$  of supernatant and  $0.001 \text{ dm}^3$  of 0.1%  $\text{H}_2\text{O}_2$ . The values obtained were presented in Bergmayer units per gram of platelet protein. One Bergmayer unit (U) determines the amount of catalase which decomposes 1 g  $\text{H}_2\text{O}_2$  per min at  $+25^\circ\text{C}$  at pH 7.0 [2].

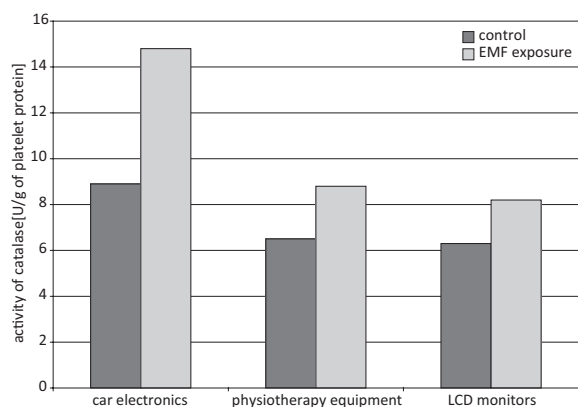
The concentration of malondialdehyde was determined by measuring the absorbance on the spectrophotometer CARY 100 BIO (VARIAN) at 532 nm wavelength vs the control sample ( $0.018 \text{ dm}^3$  PBS +  $0.0004 \text{ dm}^3$  thiobarbituric acid). The study sample was prepared adding  $0.001 \text{ dm}^3$  of 20% TCA (trichloroacetic acid) to  $0.001 \text{ dm}^3$  of blood platelet suspension at a concentration of  $1 \times 10^9/\text{cm}^3$ ; then the mixture was shaken for 1 h at  $+4^\circ\text{C}$  and centrifuged at  $4200 \times g$  at  $+4^\circ$  for 15 min. To  $0.018 \text{ dm}^3$  of the obtained supernatant,  $0.0004 \text{ dm}^3$  0.12 M thiobarbituric acid was added. The mixture was placed in a boiling water bath for 15 min. After cooling, the obtained solution was centrifuged at  $3000 \times g$  for 10 min at room temperature. The obtained results were expressed in  $\text{nmol}/10^9$  of platelets [3]. Again, 36 control and exposed samples were used.

The following statistical parameters were determined for each of the characteristics in the study groups: arithmetic mean, standard deviation and median. The obtained results were analyzed using a nonparametric Kruskal-Wallis ANOVA rank test or Friedman test equivalent to analysis of variance and a *U* Mann-Whitney test to compare the variables between the groups. The analysis of empirical distributions of the tested parameters was performed using a Shapiro-Wilk test. The value of  $p < 0.001/0.01$  or 0.05 was considered the level of significance.

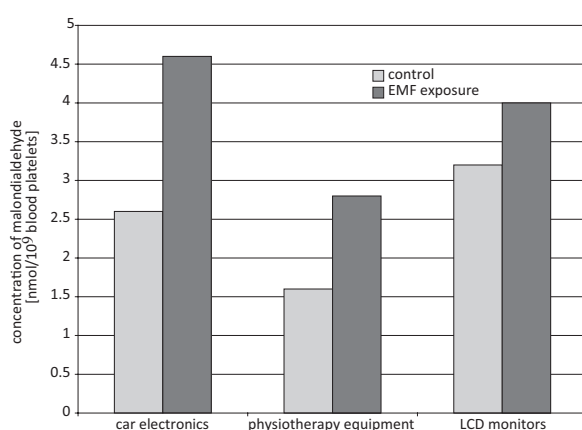
Calculations were made using the program STATISTICA PL (license number SN: SP 7105488009G 51).

## Results

The most significant changes in catalase enzymatic activity were observed in blood platelets exposed to the electromagnetic radiation emitted by car electronics (induction 0.5 mT, frequency 1 kHz) for 30 min. The activity increased by 66%



**Fig. 1.** Changes of catalase enzymatic activity under the influence of electromagnetic radiation of different parameters (n = 36)



**Fig. 2.** Changes of malondialdehyde concentration under the influence of electromagnetic radiation of different parameters (n = 36)

(from  $x = 8.9$  U/g of platelet protein to  $x = 14.8$  U/g of platelet protein) (Fig. 1).

During the 30 min exposure to electromagnetic radiation generated by physiotherapy equipment (induction 10 mT, frequency 50 Hz), catalase activity increased by 35% (from  $x = 6.3$  U/g of platelet protein to  $x = 8.8$  U/g of platelet protein).

Catalase activity increased by 30% (from  $x = 6.3$  U/g of platelet protein to  $x = 8.2$  U/g of platelet protein) after 30 min exposure of blood platelets to the radiation emitted by the field of intensity 220 V/m, frequency 1 kHz (consistent with the radiation emitted by LCD monitors).

The measurement of malondialdehyde concentration demonstrated the most significant increase (by 77%) in blood platelets after 30 min exposure to the radiation emitted by car electronics (from  $x = 2.6$  nmol/10<sup>9</sup> of platelets to  $x = 4.6$  nmol/10<sup>9</sup> of platelets) (Fig. 2).

After 30 min exposure to the field applied in physiotherapy, MDA concentration increased by 75% (from  $x = 1.6$  nmol/10<sup>9</sup> of platelets to  $x = 2.8$  nmol/10<sup>9</sup> of platelets).

Malondialdehyde concentration increased by 25% (from  $x = 3.2$  nmol/10<sup>9</sup> of platelets to  $x = 4$  nmol/10<sup>9</sup> of platelets) after 30 min exposure of blood platelets to the electromagnetic radiation generated by LCD monitors.

## Discussion

Oxidative stress reflects pro-oxidant-antioxidant imbalance. The uncontrolled increase of reactive oxygen species (ROS) developing under the influence of various external factors, among others electromagnetic radiation [4], also leads to the process of cell membrane lipid peroxidation. It is a process of oxidation of phospholipids made up of unsaturated fatty acids which leads to excessive synthesis of lipid peroxides and to the transformation of polyunsaturated fatty acids to biologically active substances.

The end products of lipid oxidation (malondialdehyde) cause disturbances of cellular membrane structures, their depolarization and loss of integrity, and consequently cause cell death. Inhibition of the activity of many enzymes, due to the activity of aldehydes, leads, among others, to the inhibition of DNA replication, transcription, DNA strand breaks and cell death [5]. MDA reacting with biomolecules building the cellular structure demonstrates its mutagenic and cytostatic properties [6]. The amount of MDA in systemic fluid may be an indicator of the occurrence of pathological processes [7].

In the study, results were obtained indicating that the highest increase of malondialdehyde (at 77%) compared to the initial values resulted from 30-min exposure to the electromagnetic field emitted by car electronics (induction 0.5 mT, frequency 1 kHz). However, the radiation emitted by physiotherapy equipment (induction 10 mT, frequency 50 Hz) also caused a great increase of malondialdehyde concentration – by 75%. The least changes were noted in the investigations concerning LCD monitors – by 25%.

The research studies conducted in other centers obtained similar results. Balci et al., in an experiment performed on rat lenticular and corneal cells, observed an increase of MDA concentration in rats exposed to radiation emitted at a distance of 20 cm from the monitor [8]. Lai et al. also proved that radiation of power frequency and induction 0.1–0.5 mT induced the occurrence of oxidative stress in rat cerebral cells [9].

In another experiment, rats were exposed to a magnetic field of 70 mT intensity once a day for 8 min for 7 successive days. The results demonstrated that the plasma level of malondialdehyde increased initially. It was found out that after

14 days of exposure to a magnetic field of frequency 40 Hz and induction 7 mT, there was a significant increase of MDA concentration in the blood of the experimental animals [10]. Also the studies on the effect of EMF of a frequency of 60 Hz and induction 2.3 mT showed an increase of MDA concentration after a 3-h exposure to mouse cerebellum [11].

Catalase (CAT) (E.C.1.11.1.6) is another parameter determining the degree of oxidative stress. As an enzyme of antioxidant defense, it prevents the accumulation of hydrogen peroxide, catalyzing the reaction of disproportionation of this compound to water and molecular oxygen. It is of importance because hydrogen peroxide, a toxic form of oxygen, is a substrate of Fenton's reaction, which leads to generation of the most reactive oxygen form – the hydroxyl radical.

In our own studies, an increase was noted of catalase enzymatic activity regardless of the source of radiation. The obtained results point to the compensatory increase of catalase activity in relation to the increase of ROS generation after exposure to electromagnetic radiation of the investigated parameters.

Similarly to the case of malondialdehyde, the most significant changes were observed under the effect of radiation generated by car electronics – by 66%. After exposure to the EMF emitted by physiotherapy equipment, the increase of this enzyme activity was 33% in relation to the control values. The least significant changes were observed during investigations of the EMF generated by LCD monitors – by 30%.

Other scientific reports describe the impact of electromagnetic radiation on the changes of cell oxygen metabolism. Catalase activity is one of its parameters. Todorović et al. also noted an increase in catalase enzymatic activity under the effect of electromagnetic radiation of 6 mT induction and 50 Hz frequency [12].

Other studies on the effect of electromagnetic radiation emitted by equipment used in magnetotherapy of the following parameters: 7 mT, 40 Hz at an exposure time of 30 min for 10 days,

demonstrated intensification of the processes of lipid membrane peroxidation in rat brain cells [13]. Most of the studies focus on the determination of the effects of electromagnetic radiation at the molecular level. This is justified by the fact that changes taking place in cells are responsible for the response of the organism as a whole. In literature, diseases are described in which oxidative stress plays a key role. These diseases include among others: multiple sclerosis, Parkinson's disease [14], atherosclerosis, rheumatoid arthritis [15], some cancers, particularly cerebral carcinomas [16] and diabetes [17].

Taking into account the numerous pathological implications of reactive oxygen species and oxidative stress reactions and the fact that we live surrounded by electromagnetic radiation, it seems to be justified to carry out further studies in this field. The results of these experiments may be helpful in the determination of standards and principles adequate to the risks that develop after exposure to EMF and also may help in the introduction of appropriate prophylaxis.

The authors concluded that catalase enzymatic activity (antioxidant defense proteins) increases significantly after 30-min exposure of human blood platelets to the EMF emitted by car electronics, physiotherapy equipment and LCD monitors. The most significant changes were observed after exposure to radiation of induction 0.5 mT and frequency 1 kHz. The measurement of malondialdehyde concentration (a lipid membrane peroxidation marker) demonstrated its increase in relation to the initial values after 30-min exposure of blood platelets to the electromagnetic radiation generated by car electronics, physiotherapy equipment and LCD monitors. The most significant changes of the concentration were noticed after exposure to radiation of induction 0.5 mT and frequency 1 kHz. The conditions (electromagnetic radiation of low frequency) used in the experiment are the cause of the observed changes in the enzymatic activity of catalase and malondialdehyde concentration, which can be indirectly related to EMF radiation in some kinds of cars, physiotherapy equipment and LCD monitors.

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