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The impact of electromagnetic radiation emitted by LCD monitors on selected blood cell counts – in vitro studies

Abstract. *There are numerous reports on the effects of electromagnetic radiation (EMR) in various cellular systems. The article presents the results of in vitro studies aimed at identifying changes in activity of the enzyme superoxide dismutase (SOD) and protective antioxidant role of vitamin E during the exposure of blood platelets to EMR (electromagnetic radiation) generated by LCD monitors.*

Streszczenie*. Istnieje wiele doniesień dotyczacych wpływu promieniowania elektromagnetycznego (EMR) na różne systemy komórkowe. W pracy przedstawiono wyniki badań in vitro mających na celu określenie zmian aktywności dysmutazy ponadtlenkowej (SOD) i antyoksydacyjnej roli witaminy E podczas ekspozycji płytek krwi na EMR (promieniowanie elektromagnetyczne) generowane przez monitory LCD (**Wpływ pola elektromagnetycznego emitowanego przez monitory LCD na wybrane elementy morfotyczne krwi – badania in vitro**).*

Keywords: electromagnetic radiation, LCD monitors, superoxide dismutase, vitamin E.
Słowa kluczowe: promieniowanie elektromagnetyczne, monitory LCD, dysmutaza ponadtlenkowa, witamina E.

Introduction

Since the experimental confirmation of the existence of electromagnetic waves and their subsequent use, the natural environment of Earth is progressively enriched by the human sources of electromagnetic radiation. Electromagnetic radiation (EMR) is characterized by mutual, orderly and repetitive impact electric and magnetic fields. The electric field (EMF) is caused by the presence of opposite electric charges, or electric voltage. In contrast, the magnetic field is caused by the movement of charge, or electric shock. Natural and manufactured electromagnetic fields accompany man everywhere: at home, at work, while traveling or leisure. Their increasingly intense occurrence is a consequence of the development of various fields of technology.

LCD monitors produce non-sinusoidal electromagnetic fields with a dominant electrical component (Fig. 1). Significant are the fields with the frequency of the impulse power supply, with suppressed RLC circuit oscillations, which act as smoothing filters for voltage ripple [2].

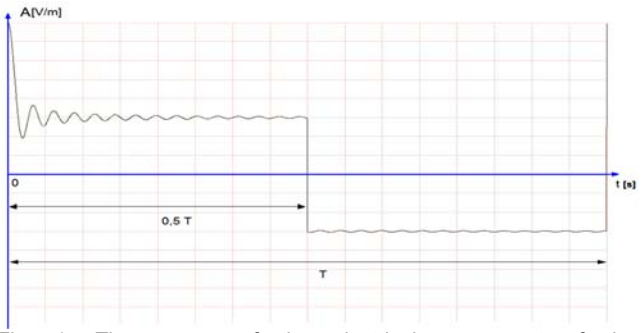

Fig. 1. The course of the electrical component of the electromagnetic field emitted by the monitor [1]

In the last years a special interest is the influence of magnetic field on the spin states of electrons and resonant absorption of electromagnetic energy. When a result of breakage of the chemical bond in the molecule produced biological couple radical, the reaction can proceed along two lines: the principle of radical recombination and separation on the basis of steam. In the latter case, free radicals are formed which may interact with the molecules of the medium in which diffuse. Selecting one of these ways depends on the relative orientation of the spins of the unpaired radical. The external magnetic field can this mutual orientation change. For biochemical processes involving pairs of radicals include, among others oxidative processes such as lipid peroxidation, or oxidative DNA damage and its repair processes [3].

Reactive oxygen species (ROS) play a crucial role in fundamental biological processes occurring in the human body. During oxidative stress occurs imbalance between the rate of formation of ROS and exercise capacity antioxidant system. Reactive oxygen species cause the oxidation of fats, proteins, DNA, and thus, may contribute to tissue damage. Toxic products of oxidation have a cytostatic effect on the cell, result in damage to cell membranes and activate apoptosis mechanisms. State of equilibrium cell is kept by enzymes and antioxidant substances such as glutathione, vitamin E, C and A or thioredoxin. These compounds allow for removal of excessive ROS cell [4].

Vitamin E (tocopherol) is among the the most powerful lipophilic antioxidants found in cell membranes and plasma lipoproteins. Vitamin E is present in an eight isoforms, the most active, encountered in humans, is associated with a cellular membrane of α-tocopherol [5]. It inhibits the oxidation of polyunsaturated fatty acids interrupting chain reactions of generating free, peroxide radical oxidized acid. Tocopherols provide structural and functional integrity of the cell membranes of intracellular organelles. The nature of the antioxidant α-tocopherol, mainly consisting of inhibition of the formation of reactive oxygen species and lipid peroxidation [6]. Mechanism to protect cells against lipid peroxidation by moving a hydrogen atom from the α-tocopherol molecule per molecule of fat, which results in oxidized form of α-tocopherol [5].

The most famous natural antioxidant enzymes include: superoxide dismutase (SOD), catalase and glutathione peroxidase. SOD (EC1.11.1.6) is an enzyme that catalyzes the dismutation reaction of superoxide anion:

$$O_2^- + O_2^- + 2H \xrightarrow{SOD} H_2H_2 + O_2$$

It occurs as the intra- and extra-cellular. Character intracellular is in the form mitochondrial of manganese in the active center (MnSOD) and cytoplasmic copper and zinc (Cu/Zn SOD). Form of extracellular (EC-SOD) is

distributed superoxide radical in the extracellular space, thereby protecting the surface vascular from the effects of superoxide radical [7,8,9].

Oxidative damage induced by reactive oxygen species is caused by increased production of superoxide anion (O_2^-) and its metabolites and/or by reduced bioavailability of antioxidant defenses. This imbalance between pro-oxidants and antioxidants gives rise to cellular oxidative stress, which plays an important role in the pathogenesis of many illnesses: cardiovascular diseases, neurodegenerative diseases or inflammations [4].

In summary, efforts to reduce oxidative stress in the cells of the body can result in improvements in the health of patients. Knowledge of the pathophysiology of oxidative stress and its biomarkers may prove to be very useful in clinical practice.

Materials and methods

Sample preparation

Pork blood was collected from a slaughterhouses during the exsanguinations of animals. It was taken to 1% ethylenediaminetetraacetic acid (EDTA). Platelets were obtained by fractionated centrifugation at 1200 rpm x g for 10 min at room temperature. As a result of the centrifugation platelet rich plasma (PRP) was obtained from the whole blood, which was carefully pulled by plastic pipette from the deposited layer of erythrocytes and transferred into polyethylene tubes. Then the obtained platelet rich plasma was centrifuged at 3000 rpm x g for 15 min. The precipitated platelets were suspended in 0,2 ml of 0,9% NaCl. The obtained suspension of blood platelets was an input research model.

Incubation of platelets with vitamin E

To the tube was dispensed test sample of the platelet suspension having a volume of 1 ml. In the shade taken 10 µl solution of vitamin E, which contains 27 µg (+) - α-tocopherol, is added to the sample, mixed. The sample was incubated at 23-250C for 30 min, then subjected to a further procedure involving platelets.

Exposure condition setting and instruments

In a laboratory stand for reconstruction of the parameters of electromagnetic radiation generated by display screens (1 kHz, 220 V/m) (Fig. 2), a flat capacitor was the source of electromagnetic field. Requirements of the TCO (The Swedish Confederation of Professional Employees) and MPR (National Board for Measurement and Testing) specifies strict conditions for the measurement of exposure. Authors measured the field by the measurement procedure on the location of points placed in front of the monitor.

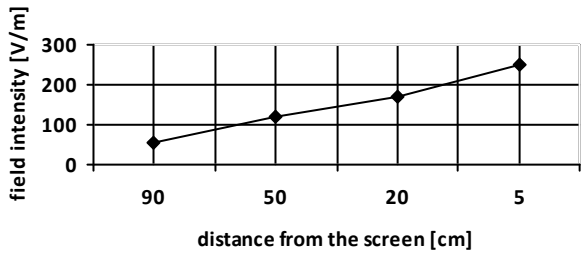

Fig. 2. The dependence of the electric field intensity on the distance from the LCD monitor screen, determined according to the TCO recommendations TCO [1]

When electromagnetic radiation of low frequency is tested the electric and magnetic components should be investigated independently. Monitors with the liquid crystal screens produce non-sinusoidal electromagnetic fields, with the dominant electric component, due to control of power semiconductor chips. Significant fields are fields with frequency the lower power consumption and voltage switching power supply, with superimposed oscillations dampened RLC circuits, which act as voltage ripple smoothing filters. The source of the signal simulating shape of the field generated by the LCD was a programmable generator Hameg 8010, which is amplified by the measuring amplifier W-320, and the source of the electric field was a flat capacitor arrangement [10]. The capacitor was formed by two circular copper plates positioned over and under a plastic support in which 8 polyethylene tubes containing the tested preparation were inserted into holes made symmetrically on the circumference of the circle the diameter of which was smaller than that of the capacitor plates so that the electrical component of the field acting on the tubes was homogeneous in nature. The tested preparation was placed in polyethylene tubes, each containing 0,2 ml of the preparation. The temperature in the laboratory stand was on the same level all the time and it was +24/+25^{0}C. Preserving constant conditions of the environment the preparation was exposed to the activity of the electromagnetic field of 1 kHz frequency and 220 V/m intensity (corresponding to a distance of 15 cm from the monitor) for 30 and 60 min. The exposure of the platelets to the radiation was done on the day they were collected from the slaughterhouses.

Measurement of antioxidant activity of superoxide dismutase (Cu, Zn-SOD) (SOD-1) (EC.1.15.1.1.)

This parameter of oxidative stress were measured before and immediately after the exposure. The study samples were obtained by adding 0,2 cm^3 of platelet suspension at the concentration of 1x10^9/cm^3, 0,8 cm^3 redestilled water cooled to +4^{0}C and 0,5 cm^3 of 96% C_2H_5OH and 0,25 cm^3 chloroform. The obtained mixture was shaken for 4 min. and then centrifuged at 4200 x g at +4^{0}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 together with 2,6 cm^3 0,05M carbonate buffer of pH 10,2 and 0,2 cm^3 of adrenaline. The blind test did not contain supernatant, the carbonate buffer was used instead. 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/min on a rectilinear segment of adrenochrome formation at +25^{0}C at 480 nm is defined as a unit of SOD activity [11,12]. It was used 30 control and exposed samples.

Spectrophotometer T60 VIS the company OMC Envag was used for the measurement of superoxide dismutase activity at 480 nm wavelength. Absorbance in the control and study samples was measured every minute at +25^{0}C for 10 min.

Statistical analysis

The following statistical parameters were determined for each characteristics in the study groups: arithmetic mean, standard deviation, median, minimum, maximum. The obtained results were analyzed using a nonparametric Kruskal-Wallis Anova rank test equivalent to analysis of variance and U Mann-Whitney test to compare the variables between the groups. The value of $p<0,05$ was considered the level of confidence. Calculations were made using the program STATISTICA. PL (license number S/N: JPZP601E504326AR-9).

Results

There are numerous reports on the effects of electromagnetic radiation (EMR) in various cellular systems. Mechanisms of adverse effects of EMR indicate that

reactive oxygen species (ROS) may play a role in the biological effects of this radiation.

In the in vitro studies the superoxide dismutase concentration in blood platelets increases significantly ($p<0,05$) compared to control values after 30 minutes of exposure to EMF of 220 V/m intensity and 1 kV/m frequency (from md=1,34 to md=2,44) and after 60 minutes of exposure to EMF of 220 V/m intensity and 1 kV/m frequency (from md=1,34 to md=2,50) (Table 1).

Table 1. Statistical analysis (descriptive) in each study groups

variable	arithmetic mean ($\bar{x}$)	median (md)	minimum (min)	maximum (max)	standard deviation (SD)
control	1,26	1,34	0,15	2,04	0,56
control + vit E	0,88	0,58	0,18	2,21	0,66
exposure 30min	2,34	2,44	1,12	3,60	0,64
exposure 30min + vit E	1,39	1,15	0,64	2,70	0,67
exposure 60min	2,54	2,50	1,38	4,37	0,82
exposure 60min + vit E	0,43	0,40	0,13	0,79	0,20

The concentration of superoxide dismutase significantly decreases ($p<0,05$) in the blood sample unexposed to EMF with vitamin E in comparison with the unexposed sample (from md=1,34 to md=0,58). There is a strong relationship between the blood sample unexposed to EMF with vitamin E in comparison with the unexposed sample ($Z_{correction}$=2,09596; $p<0,05$) (Table 2, Fig 3).

Table 2. Statistical analysis (dependence) in each study groups

Statistical analysis	Result	
Test Kruskala-Wallisa	H (5, N=150) = 92,94137; p<0,05	
Test U Manna-Whitneya	control vs control + vit E	$Z_{correction}$ = 2,09596; p<0,05
	control vs exposure 30min	$Z_{correction}$ = -4,70632; p<0,05
	control vs exposure 60min	$Z_{correction}$ = -4,95921; p<0,05
	exposure 30min vs exposure 30min + vit E	$Z_{correction}$ = 4,08587; p<0,05
	exposure 60min vs exposure 60min + vit E	$Z_{correction}$ = 6,05645; p<0,05

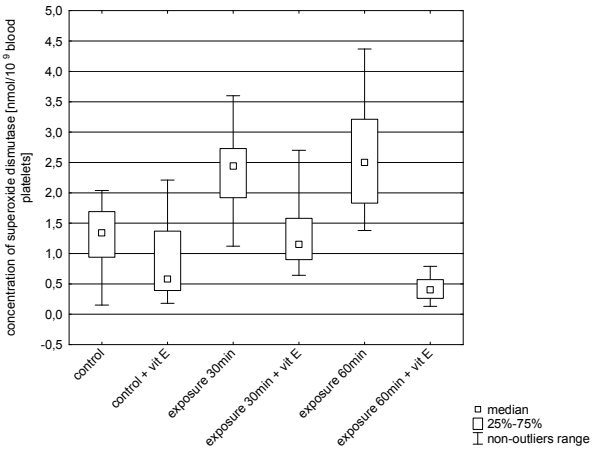

Fig.3. Concentration of superoxide dismutase in each study groups

SOD concentration significantly decreases ($p<0,05$) in the blood sample exposed to EMF for 30 min, to which vitamin E was added as compared with the sample exposed for the same period of time without vitamin E (from md=2,44 to md=1,15). There is a strong relationships between the blood sample exposed to EMF for 30 min, to which vitamin E was added as compared with the sample exposed for the same period of time without vitamin E ($Z_{correction}$=4,08587; $p<0,05$) (Table 2, Fig 3).

Superoxide dismutase concentration statistically significant decreases in the blood sample exposed to the EMF for 60 min, to which vitamin E was added as compared with the sample exposed for the same period of time without vitamin E (from md=2,50 to md=0,40). There is a strong relationship between the blood sample exposed to the EMF for 60 min, to which vitamin E was added as compared with the sample exposed for the same period of time without vitamin E ($Z_{correction}$=6,05645; $p<0,05$) (Table 2, Fig 3).

Discussion

Electromagnetic field is a space in which electric and magnetic penetrate each other. It accompanies us in every area of life. As technology improves every year there are electromagnetic devices. In common use are systems broadcasting, radiocommunications fixed and mobile, radiolocation and navigation, power industrial equipment, medical diagnostic equipment and therapeutic as well as power tools and household electrical appliances. The electromagnetic fields generated by these systems or devices operating in close proximity may significantly worsen the conditions of human existence. However, the excess active emitters can be harmful to the human body [3].

The effects of devices emitting electromagnetic field on human health have become the subject of intense research among scientists due to the rapid increase in their use. Based on Kuybulu et al. study, it is thought that chronic pre- and post-natal period exposure to wireless internet frequency of EMF may cause chronic kidney damages; staying away from EMF source in especially pregnancy and early childhood period may reduce negative effects of exposure on kidney [13].

The number of people complaining about different symptoms that may be associated with exposure to electromagnetic fields has increased rapidly during past years. The results of Kucer et al. has shown that users of mobile phone and computer more often complained of headache, joint and bone pain, hearing loss, vertigo/dizziness, tension-anxiety symptoms according to time of daily usage ($p<0,05$). Moreover in users of mobile phone and computer, women significantly ($p<0,05$) complained more often of headache, vertigo/dizziness, fatigue, forgetfulness and tension-anxiety than men [14]. In turn, the studies Mortazavi observed a significant association between cordless phone use and difficulties in concentration ($p<0,05$) or attention disorders ($p<0,05$) [15].

In addition, interactions PEM biological systems under certain conditions can cause oxidative stress, or imbalance in pro - antioxidant, during which proteins antioxidant defenses (eg. superoxide dismutase, catalase) can not keep up compensate for an uncontrolled increase in reactive oxygen species, which react with the components cells. These reactions may have undesirable and even dangerous for the body health effects and cause disease free radical. For pathological implications of reactive oxygen species and oxidative stress include inter alia: multiple sclerosis, Parkinson's disease, in which the observed increased production of ROS by phagocytes blood and increasing the level of MDA in plasma [16]. Research also demonstrated that ROS and antioxidants affect the replication of HIV in the body. Many authors also believes that oxidative stress brain may underlie or at least accompany schizophrenia [17]. In children, oxidative stress underlies diseases such as hydrocephalus, Down syndrome, autism, brain injuries and brain tumors [18].

Therefore, researchers are trying to deal with the current problem using natural anti-oxidant agents that can reduce the production of reactive oxygen species generated after the exposure to EMF radiation [19]. Among these agents are vitamin E and Silymarin which are effective anti-oxidant and free radical scavenger agent [19,20]. Vitamin E (α-tocopherol) is found in virtually all cell membranes, especially in the inner mitochondrial membrane, the site of the electron-transport system. Vitamin E is a lipid – soluble chain – antioxidant high protects the biological membranes from lipid peroxidation [19].

The protective effect of α-tocopherol was also seen in our study. There is a strong correlation between the level of SOD in the control samples compared with blood samples with vitamin E. As confirmed by the results of its own Vitamin E strong impact on the free radicals resulting in a decrease SOD, ie. control: md=1,34 vs control+vitamin E: md=0,58. Especially significant relationship is shown on the exposure time of 60 minutes to EMF of 220 V/m intensity and 1 kV/m frequency in samples without and after addition of vitamin E: md=2,50 vs md=0,40.

Primary antioxidants such as superoxide dismutase are our first and most important line of defense against highly reactive, potentially destructive oxygen-derived free radicals. Researchers believe that SOD decreases with aging and evidence suggests that boosting falling SOD levels may help guard against disease and extend life span [21].

How to show the results of Hidisoglu, indicated that long-term EMF could have an adverse effect on oxidant/antioxidant status [22]. In studies Irmak, serum SOD activity increased, and serum NO levels decreased in EMR-exposed animals compared to the control group [23].

Many studies confirm the negative effect of EMF on the metabolism aerobic of cells. In one of the experiments on the harmfulness of mobile phones, where the platelets treated with PEM at a frequency of 1800 MHz and a power of 0,2 W 1 and exposure time of 5 minutes was detected increase in the concentration of reactive oxygen species (ROS) [24]. Increased production of ROS after combined radiation (930 MHz, 1,5 WKG SAR-1) and iron ions has been noted during the course of experimental tests on the model lymphocytes in the rat [25]. Furthermore, induction peroxidation of lipid accompanied by a reduction of superoxide dismutase (SOD), myeloperoxidase (MPO) and glutathione peroxidase (GSH-Px) in various organs such as the kidneys and liver of rats guinea pigs after exposure to radiowaving radiation [26,27,28].

On the other hand, the results study's Topal et al. show that a 900-MHz EMF applied in the prenatal period caused oxidative stress and pathological alterations in the liver in the postnatal period. Biochemical analyses showed that malondialdehyde and superoxide dismutase values increased and glutathione levels decreased in the experimental group compared with the group not exposed to an electromagnetic field [29]. A similar relationship was observed in the present experiment. The electromagnetic field to which it is exposed to the test samples resulted in activation of SOD for disposal of free radicals. In the in vitro studies the superoxide dismutase concentration in blood platelets increases significantly ($p<0,05$) compared to control values after 30 minutes of exposure to EMF of 220 V/m intensity and 1 kV/m frequency (from md=1,34 to md=2,44) and after 60 minutes of exposure to EMF of 220 V/m intensity and 1 kV/m frequency (from md=1,34 to md=2,50). While according to Hanci et al., exposure to 900 MHz EMF during the prenatal period can cause pathological and biochemical changes that may compromise the development of the male rat thymus and spleen. Increased malondialdehyde and glutathione levels were observed in splenic tissue of rats exposed to EMF, while a significant decrease occurred in superoxide dismutase values compared to controls [30]. In turn another relationship was observed in the study Odaci where malondialdehyde, superoxide dismutase, catalase and glutathione values in the group rats were exposed to 900 MHz EMF (1 h/30 day) decreased significantly compared to those of the control group [31]. Whereas, as observed in the studies Li et al., compared with the control group, activities of serum alanine aminotransferase and aspartate aminotransferase and concentrations of serum, liver, and spleen metabolism of lipid peroxidation (MDA) in the 10- and 20-mT PEMF groups were significantly increased. The activities of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in the serum, liver, and spleen and concentrations of serum immunoglobulins were significantly decreased [32].

The changes of enzymatic activity of superoxide dismutase in our study may indicate the negative effect of the used radiation and the protective antioxidant role of vitamin E. The presented results of these and other authors' study should suggest an important preventive role of vitamins A, C and E to protect against the effects of electromagnetic radiation which we are exposed to in everyday life.

Conclusion

1. The study demonstrated that the exposure to EMF emitted by LCD monitors changes the superoxide dismutase (SOD) concentration – a marker of lipid peroxidation in blood platelets. After 30 and 60-minute irradiation of field of 220 V/m intensity the SOD concentration increases relatively to the control value.
2. The findings indicate that exposure to electromagnetic radiation of 1 kHz frequency and 220 V/m intensity may cause adverse effects within blood platelets oxygen metabolism and thus may lead to the organism physiological dysfunction.
3. The observed changes in the SOD concentration in the pork blood samples after the addition of vitamin E may indicate the protective antioxidant role of vitamin E.

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