Antioxidant response of vitamin A during the exposure of blood platelets to electromagnetic radiation generated by LCD monitors - in vitro study

Małgorzata Lewicka¹*, Magdalena Zawadzka¹, Gabriela Henrykowska¹, Maciej Rutkowski², Krzysztof Pacholski³, Andrzej Buczyński¹

¹ Department of Epidemiology and Public Health, Military Medical Faculty, Medical University, ul. Żeligowskiego 7/9, 91-752 Łódź, Poland
² Department of Military Toxicology and Radiological Protection, Military Medical Faculty, Medical University, Łódź, Poland
³ Institute of Electrical Engineering Systems, Faculty of Electrical, Electronic, Computer and Control Engineering, Technical University, Łódź, Poland

* Corresponding author: Małgorzata Lewicka; Tel. +48 (42) 639 32 60; E-mail: malgorzata.lewicka@umed.lodz.pl

ABSTRACT

The article presents the results of in vitro studies aimed at identifying changes in activity of the enzyme superoxide dismutase (SOD-1) as a parameter of oxidative stress and protective antioxidant role of vitamin A during the exposure of blood platelets to electromagnetic radiation (EMR) generated by LCD monitors. Blood platelets were exposed to an electromagnetic radiation for 30 min. and 60 min. generated by monitors, which is characterized by parameters: 1 kHz frequency and 220 V/m intensity. The enzymatic activity of SOD-1 increases significantly compared to control values after 30 min. of exposure to EMR (from 2523.39 U/g protein to 3896.15 U/g protein), and decreases after 60 min (to 2846.58 U/g protein). A significant decrease in enzyme activity after the addition of vitamin A was noticed (to 1569.54 U/g protein). In samples exposed for 30 min. the SOD activity was significantly increased by addition of vitamin A and decreases after 60 min. Changes in enzymatic activity of SOD-1 dependent on exposure time and application of vitamin A suggest an important preventive role of vitamin A to protect against the effects of EMR which we are exposed to in everyday life.

Keywords: Electromagnetic radiation; LCD monitors; Superoxide dismutase; Vitamin A; Antioxidants; Oxidative stress.

1. INTRODUCTION

For several dozen years, power tools have become an integral part of life for most societies. Any such device emits electromagnetic radiation that as a new environmental factor drew researchers’ attention starting with the 1960s. After years numerous reports about its harmfulness to living organisms appeared and attempts to limit the negative consequences of its effects were made. Prophylaxis for electromagnetic radiation (EMR) can rely on the norm creation in the particularly
dangerous places and antioxidant prevention aimed at alleviating the effects of oxidative stress, one of the most dangerous effects of electromagnetic radiation.

Oxidative stress is a state of disturbed balance between oxidative processes that induce the formation of reactive oxygen species (ROS) and counteracting antioxidant defense system. ROS oxidizing proteins, lipids, DNA contribute to cellular damage and consequently to apoptosis. The state of pro-oxidant-antioxidant balance is maintained by the activity of enzymes such as superoxide dismutase, catalase, glutathione peroxidase, and other low molecular weight substances, for example melatonin, vitamin A (used in medicine as tretinoin), C (ascorbic acid) and E (tocopherol) [1]. The reactions of the low molecular weight antioxidants with ROS are less specific than the antioxidant enzymes, causing these compounds more universal protectors, performing several functions. They act as a second line of defense degrading ROS, which are not removed by superoxide dismutase and catalase. The biological role of superoxide dismutase (SOD-1) (EC 1.15.1.1) consist in removal of superoxide anion radical by dismutation into oxygen and hydrogen peroxide: 

$$O_2^{•−} + O_2^{•−} + 2H^+ \rightarrow H_2O_2 + O_2$$

In turn, the enzyme catalase (EC 1.11.1.6) prevents the build-up of hydrogen peroxide catalyzing the disproportionation reaction of this compound.

Oxidative stress underlies many pathological conditions and diseases. The pathological implications of the reaction of reactive oxygen species and oxidative stress include, inter alia, multiple sclerosis, atherosclerosis, rheumatoid arthritis, Parkinson's disease [2], Alzheimer's disease [3], diabetes, in which increased ROS production by phagocytes and elevated plasma MDA level were observed [4]. Other studies also proved that ROS and antioxidants stimulate HIV replication in an organism [5].

There is also evidence that ROS, produced mainly by activated neutrophils infiltrating the wound or to the locus in which the inflammation occurred, increased expression of some proto-oncogenes and may also be mediators of inflammation cocarcinogenic action [6].

In addition, studies have shown that tumors are often characterized by decreased activity of superoxide dismutase Cu, ZnSOD, where the activity of another kind of dismutase - MnSOD lowers as a rule [1].

Studies carried out on the molecular level are justified by the fact that changes taking place in cells are responsible for the response of the organism as a whole. There are many reports focused on the influence of electromagnetic radiation on the oxidative metabolism of cells: increased lipid peroxidation [7, 8] and changes in the activity of antioxidant enzymes in various cells and tissues [9, 10]. Additionally, the effect of oxidative stress caused by electromagnetic radiation is confirmed by numerous studies [11, 12].

Study of Agarwal et al. reported that chronic exposure to electromagnetic radiation reduced the enzymatic activity of superoxide dismutase, glutathione peroxidase and catalase, and increased the lipid peroxidation [13]. The harmfulness of electromagnetic radiation emitted by the LCD monitors was showed by the epidemiological studies of Korpinen et al.

The respondents had skin symptoms when they stayed in front of a computer screen for a long period [14]. Results of other studies indicate that computer users more often complained of headaches, bones and joint pain, hearing loss, vertigo/dizziness, tension - anxiety symptoms depending on the time of daily usage [15].

Among the biochemical studies conducted on the effects of electromagnetic field (EMF) emitted by monitors, Balci’s et al. experiments conducted on corneal and lens tissue of rats reported harmful effects. The results of these studies indicated that this factor may induce oxidative stress manifested in an increase of the MDA concentration and activity of antioxidant enzymes [16].

Considering the above data, the authors of this study attempted to determine the effect of vitamin A on the oxidation - reduction reaction occurring in the blood platelets under the influence of electromagnetic radiation generated by LCD monitors. The aim of this study was to determine the applicability of this antioxidant vitamin as prophylactic action, shielding the body from the harmful effects of EMF.
2. MATERIALS AND METHODS

2.1. 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.

2.2. Incubation of platelets with vitamin A

An ethanolic solution of vitamin A containing 3 mg of retinol (cat. no. R7632-25MG) in a volume of 10 ml was used in this study. 2 µl of this solution was added to 0.2 ml of a suspension of blood platelets, avoiding bright light. The sample was incubated in a dark place for 30 min., and then subjected to a further procedure.

2.3. Exposure condition setting and instruments

In a laboratory stand designed for reconstruction of the parameters of electromagnetic radiation generated by display screens (1 kHz, 220 V/m), 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.

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. 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°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.

2.4. 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 cm³ of platelet suspension at the concentration of 1x10⁹/cm³, 0.8 cm³ redistilled water cooled to +4°C and 0.5 cm³ of 96% C₂H₅OH and 0.25 cm³ chloroform. The obtained mixture was shaken for 4 min. and then centrifuged at 4200 x g at +4°C for 10 min. After centrifugation, the enzyme remained in the upper layer of the suspension. Then 0.2 cm³ of supernatant was transferred into glass tubes together with 2.6 cm³ 0.05M carbonate buffer of pH 10.2 and 0.2 cm³ 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°C at 480 nm is defined as a unit of SOD activity [17]. It was used 30 control and exposed samples.

Spectrophotometer T60 VIS firmy 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°C for 10 min.

2.5. Statistical analysis

The following statistical parameters were determined for each characteristics in the study groups: arithmetic mean, standard deviation, median, minimum, maximum, skewness coefficient. All data were presented as median ± SD. The obtained results were analyzed using a non-parametric Kruskal-Wallis Anova rank test equivalent to analysis of variance and Mann-Whitney U 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 (Table 2).

3. RESULTS

Each of 30 sample blood was divided into 6 fractions, each of them distributed in a different experimental group: unexposed to radiation, unexposed + vitamin A, exposed for 30 min., exposed for 30 min + vitamin A, exposed for 60 min., exposed for 60 min. + vit. A. In each sample the level of SOD-1 activity were determined. In the in vitro studies the enzymatic activity of superoxide dismutase 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 2523.39 U/g protein to 3896.15), and then the activity decreases (measured after 60 min.), being higher (not statistically significant p > 0.05) compared to initial values (from 2523.39 U/g protein to 2846.58 U/g protein). The activity of SOD significantly decreases (p < 0.05) in the blood sample unexposed to EMF with vitamin A in comparison with the unexposed sample (from 2523.39 U/g protein to 1569.54 U/g protein).

The activity of SOD significantly increases (p < 0.05) in the blood sample exposed to EMF for 30 min., to which vitamin A was added as compared with the sample exposed for the same period of time without vitamin A (from 3896.15 U/g protein to 7442.87 U/g protein). The activity of superoxide dismutase decreases (not statistically significant) in the blood sample exposed to the EMF for 60 min., to which vitamin A was added as compared with the
sample exposed for the same period of time without vitamin A (from 2846.58 U/g protein to 2166.24 U/g protein) (Table 1, Figure 1).

Table 2. Statistical analysis of the enzyme activity of superoxide dismutase (SOD-1) in blood platelets treated with electromagnetic radiation dependent on exposure time and application of vitamin A (n = 30).

<table>
<thead>
<tr>
<th>Kruskal-Wallis Anova rank test</th>
<th>H = 91.0042 p&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Test Z^I,III_Mann-Whitney</td>
<td>Z = -3.72 p&lt;0.05</td>
</tr>
<tr>
<td>2) Test Z^I,IV_Mann-Whitney</td>
<td>Z = -1.06 p&gt;0.05</td>
</tr>
<tr>
<td>3) Test Z^II,III_Mann-Whitney</td>
<td>Z = 3.34 p&lt;0.05</td>
</tr>
<tr>
<td>4) Test Z^III,IV_Mann-Whitney</td>
<td>Z = -3.91 p&lt;0.05</td>
</tr>
<tr>
<td>5) Test Z^IV,VI_Mann-Whitney</td>
<td>Z = 1.69 p&gt;0.05</td>
</tr>
</tbody>
</table>

H - value of the Kruskal-Wallis Test; Z - value for pair of variables: 1) correlation between control (I) and exposure to EMR, 30 min. (III); 2) correlation between control (I) and exposure to EMR, 60min. (V); 3) correlation between control (I) and control + vit. A (II); 4) correlation between exposure to EMR, 30 min. (III) and exposure to EMR, 30 min. + vit. A (IV); 5) correlation between exposure to EMR, 60 min. (V) and exposure to EMR, 60 min. + vit. A (VI).

4. DISCUSSION

Despite numerous inconsistencies, decades of research on the effects of electromagnetic radiation proved the negative effect of this factor on the health of living organisms e.g., on the cardiovascular system [18], nervous system [19], as well as the formation of tumors [20]. Studies conducted at the cellular level focused on the analysis of individual parameters of oxidative stress, ie. free radicals generation, the enzymatic activity of superoxide dismutase, catalase, glutathione peroxidase, or a concentration of malondialdehyde - a marker of lipid peroxidation also indicate a negative impact of EMF.

Research on the effects of electromagnetic field of 1000 Hz frequency, and a magnetic induction of 0.5 mT on the enzymes antioxidant defense of platelets also showed reduction of superoxide dismutase activity after both the 30- and 60- and 90-minute exposure [21].

In another study, authors have found that vertical and horizontal application of ELF electric fields in the range of 1.35, 1.5, and 1.8 kV/m increased SOD levels as compared to the controls (p<0.05) and to applied electric fields of 0.3, 0.6, 0.8, and 1 kV/m [10].

Our study demonstrated that the exposure to EMF emitted by LCD monitors changes the activity of the superoxide dismutase enzyme in blood platelets. After 30-minute irradiation of field of 220 V/m intensity the enzyme activity increases relatively to the control value, and then decreases (measured after 60 min.).

As a result of EMFs effect, an increase in generation of free radicals both in the cell membrane platelet blood cells and organelles is induced - as confirmed by the above-mentioned research, including their own authors [22]. This can cause changes in SOD enzyme activity due to the increased concentration of free radical substrates.

Vitamin A is a general term that refers to fatsoluble compounds from the group of retinoids. The active form of vitamin A is retinol that is similar in structure and biologic activity. The carotenoids (most commonly beta-carotene) are the precursors of vitamin A (retinol). The role of vitamin A as an antioxidant is debatable. The carotenoids such as beta-carotene have in recent years received more attention from the scientific community because of the harmful role they may play as pro-oxidants [23]. Studies have shown that high dose of beta-carotene increases the incidence of lung cancer and increases mortality among smokers [24].

Additionally, the results of large, controlled trials of an intervention of beta-carotene supplementation did not support the detected beneficial associations or a role for supplemental beta-carotene in lung cancer prevention; instead, they provided striking evidence for its adverse effects among smokers [25]. Despite these discrepancies, vitamin A is known to help repair damaged tissue and therefore may be beneficial in counter-acting free radical damage [26].

The conclusion is that beta-carotene may serve as an antioxidant or as a prooxidant, depending on its intrinsic properties as well as on the redox potential of the biological environment in which it acts.
Among the many studies in the field of antioxidant role leveling effects of electromagnetic radiation of vitamins those that relate to vitamins C and E are the most numerous.

The results of the study of Jelodar et al. suggest that radio waves lead to oxidative stress in testis tissue and vitamin C via antioxidant role improved antioxidant enzymes level and decreased lipid peroxidation [27].

Results of Al-Damegh study indicate that the electromagnetic radiation from conventional cellular phone had a negative impact on the oxidant and antioxidant status in rat blood and testicular tissue. This finding also indicated the possible role of vitamins C and E in mitigating the oxidative stress imposed on the testes and restoring normality to the testes [28].

Karsiloglu et al. examining the protective effect of vitamin E on the occurrence of gamma irradiation-induced cataract in rats lens, showed that this vitamin’s antioxidant role is to act by reducing oxidative stress and thus, the incidence of cataracts. It has been shown among others that vitamin E increases enzymatic activity of superoxide dismutase and glutathione peroxidase [29].

Studying the available literature, we can find publications about the antioxidant role of carotenoids, especially beta-carotene (a precursor of vitamin A), but little is related to vitamin A. Moreover, the available publications relate mainly to the protective role of these compounds in UV radiation protection.

A study by Stahl et al. investigated the antioxidant effect of carotenoids and tocopherols based on their ability to scavenge ROS generated during photooxidative stress. The antioxidants used in this study provided protection against erythema in humans and may be useful for diminishing the sensitivity to ultraviolet light [30].

The changes in the activity of superoxide dismutase in the pork blood samples after the addition of vitamin A were observed in the present study. When comparing samples of control material - not exposed to EMF (control vs control + vit. A) a significant decrease in enzyme activity after the addition of this vitamin was noticed. Vitamin A acts as an antioxidant by scavenging existing free radicals (arising due to natural metabolism), which probably contributes to the decrease in the amount of free radical substrates for the operation of SOD, causing a decrease in its activity.

In the blood samples exposed to EMF for 30 minutes the SOD activity was significantly increased by addition of vitamin A. In this case it seems that vitamin A as an auxiliary antioxidant action of cellular enzymes contributes to increasing their activity.

Whereas, after the 60-minute exposure to EMF, SOD activity decreases after adding vitamin A (exposed for 60 min. vs. exposed for 60 min. + vit. A). In this case, after prolonged exposure, a depletion of the enzymatic activity of SOD follows and thus the antioxidant activity of vitamin A also decreases. As a result the generation of free radicals may increase leading to cellular damage, for example in the intensified process of lipid peroxidation in cell membranes, which can be expressed by the above-mentioned increase of malondialdehyde (MDA) concentrations - marker of peroxidation changes.

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 A.

The presented results suggest an important preventive role of vitamins A, C and E to protect against the effects of electromagnetic radiation.

AUTHORS’ CONTRIBUTION

Conception and design, Study supervision: AB; Development of methodology: MR, KP; Acquisition of data: ML, GH; Analysis and interpretation of data: ML, MZ; Writing, review and/or revision of the manuscript: ML; Administrative, technical, or material support: MR. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

REFERENCES

8: 809-814.
3. Davanipour Z, Tseng CC, Lee PJ, Sobel E. A case-  
dcontrol study of occupational magnetic field  
and Alzheimer's disease: results from the  
California Alzheimer's Disease Diagnosis and  
4. Beisswenger PJ, Szwergold BS, Yeo KT. Glycated  
5. Israel N, Gougerot-Pocidalo MA. Oxidative stress in  
human immunodeficiency virus infection. Cell Mol  
6. Gromadzińska J, Wąsowicz W. The role of reactive  
oxidant oxygen species in the development of  

245.
7. Akpinar D, Ozturk N, Ozen S, Agar A, Yargicoglu P.  
The effect of different strengths of extremely  
low-frequency electric fields on antioxidant status,  
lipid peroxidation, and visual evoked potentials.  
8. Bilgici B, Akar A, Avci B, Tuncel OK. Effect of  
900 MHz radiofrequency radiation on oxidative  
Effect of extremely low frequency magnetic field on  
antioxidant activity in plasma and red blood cells in  
10. Guler G, Turozer Z, Seyhan N. Electric field effects  
on guinea pig serum: the role of free radicals.  
11. Yokus B, Akdag MZ, Dasdag S. Extremely low  
frequency magnetic fields cause oxidative DNA  
12. Lewicka M, Henrykowska GA, Pacholski K,  
Szczęsny A, Dzierdziczak-Buczyńska M, Buczyński A. The  
impact of electromagnetic radiation of  
different parameters on platelet oxygen metabolism-

phones and male infertility: a review of recent  
innovations in technology and consequences. J Int  
symptoms associated with using mobile phones and  
15. Kucer N, Pamukcu T. Self-reported symptoms  
associated with exposure to electromagnetic fields: a  
16. Balci M, Namuslu M, Devrim E, Durak I. Effects of  
computer monitor-emitted radiation on oxidant/  
antioxidant balance in cornea and lens from rats.  
17. Misra H, Fridovich J. The role of superoxide anion  
in the autoxidation of epinephrine and a simple  
18. Hamburger S, Logue JN, Silverman PM.  
Occupational exposure to non-ionizing radiation and  
an association with heart disease: an exploratory  
19. Savitz D, Checkoway H, Loomis D. Magnetic field  

display and neurodegenerative disease mortality  
I, Schlaefer K, et al. Cellular phones, cordless  
phones and the risk of glioma and meningioma. Am  
21. Buczyński A, Pacholski K, Talar J, Dzierdziczak-  
Buczyńska M, Jeromino A. Evaluating the activity  
of catalase (CAT) and superoxide dismutase (SOD-
1) in blood platelets exposed to electromagnetic  
22. Buczyński A, Pacholski K, Dzierdziczak-Buczyńska  
M, Lewicka M, Henrykowska G. Changes in free  
radicals generation in blood platelet exposed to  
electromagnetic radiation emitted by monitors. Pol  
23. Volpe S. Vitamins and minerals for active people.  
In: Sports nutrition: a guide for the professional  
working with active people. Rosenbloom CA, ed.  
3rd edn. American Dietetic Association, Chicago,  
III; 2000: 63-93.
24. Omenn GS. Chemoprevention of lung cancers:  
lessons from CARET, the beta-carotene and retinol  
efficacy trial, and prospects for the future. Eur J  
25. Albanes D. Beta-carotene and lung cancer: a case  
26. Ross AC. Vitamin A. In: Modern nutrition in health  
and disease. Baltimore: Williams & Wilkins, 8th  
27. Jelodar G, Nazifi S, Akbari A. The prophylactic  
effect of vitamin C on induced oxidative stress in rat  
testis following exposure to 900 MHz radio

28. Al-Damegh MA. Rat testicular impairment induced by electromagnetic radiation from a conventional cellular telephone and the protective effects of the antioxidants vitamins C and E. Clinics. 2012; 67(7): 785-792.
