The assessment of oxygen metabolism selected parameters of blood platelets exposed to low frequency magnetic radiation in cars – in vitro studies

Buczyński A[1], Pacholski K[2], Dziedziczak-Buczyńska M[1], Henrykowska G[1], Jerominko A[1]

[1] Health Promotion and Education Department, Medical University Łódź, Poland
[2] Institute of Circuit Theory, Measurement Science and Materials Science, Technical University of Łódź, Poland

Abstract

Purpose: **The aim of the study was to determine how free radicals generation in blood platelets exposed to electromagnetic field (EMF) occurring in cars affects the process of these morphotic elements cell membranes phospholipid peroxidation.**

Material and methods: **The suspension of human blood platelets was exposed to EMF of proper characteristics in a specially arranged research stand. After 30, 60 and 90 min exposure of the platelet specimen to EMF, free radicals generation was measured with chemiluminescence and malondialdehyde concentration according to Placer et al. method. The obtained results were compared with the control values.**

Results: **The increase of free radicals generation was observed after 30 and 90 min exposure of platelets to magnetic field. Malondialdehyde reached the highest values also after 30 and 90 min exposure of the platelets to EMF as compared to the control.**

Conclusions: **The increase in oxygen reactive species generation under the effect of exogenic magnetic radiation as well as proportional intensification of the peroxidation process determined on the basis of malondialdehyde concentration (the marker of this phenomenon) point to the platelet sensitivity to the investigated environmental factor.**

Key words: electromagnetic field (EMF), blood platelet, free radicals (FR), malondialdehyde (MDA), car.

ADDRESS FOR CORRESPONDENCE:
Prof. dr hab. n. med. Andrzej Buczyński
Zakład Edukacji Zdrowotnej i Promocji Zdrowia UM w Łodzi
90-752 Łódź, ul. Żeligowskiego 7/9

Received 21.02.2005 Accepted 07.02.2005

Introduction

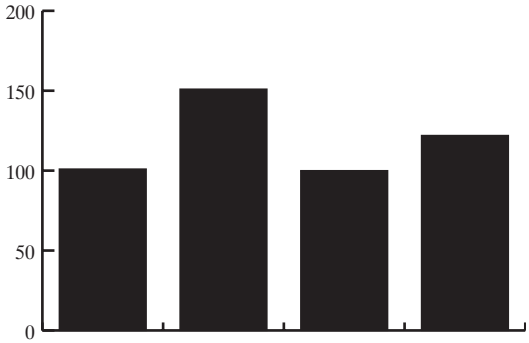
The life environment of a man has undergone significant changes as the result of rapid technological development. At present, the biosphere is formed by overlapping the natural conditions of the environment with the factors being the effect of evolutionary technological and industrial transformations. Electromagnetic smog may be an example of a confrontation of natural phenomena with these artificially produced.

The natural magnetic field of the earth amounts dependently on the place of measurement from 40 to 60 μT. Electromagnetic field from artificial emitters overlaps radiation. Most frequently environmental and occupational exposure concerns radio and microwave frequencies (100 kHz – 3 GHz) and power frequencies (50, 60 Hz). Numerous studies have been carried out describing the effect of the above mentioned electromagnetic field on a human organism [1,2].

In our environment, EMF of different parameters than those mentioned is more and more frequently observed. Significant development of motorization resulted in the increase of the number of subjects exposed environmentally and occupationally to electromagnetic radiation emitted by car electronics. A modern car vehicle, apart from conventional electronic equipment i.e. ignition system, feed system and lightning and signalling installations, is equipped in electronic systems increasing the comfort and safety of driving (e.g. Airbag, ABS, ASR and others) [3]. Development of the vehicle electronic equipment requires the development of electrical installation. Modern electrical installation due to its task, covers with a dense net practically the whole inner side of the car floor and instrument panel. The impulse interference field from electronics is characterised by magnetic component of induction not exceeding 1 mT and frequency form 0.5 to 3 kHz [4].

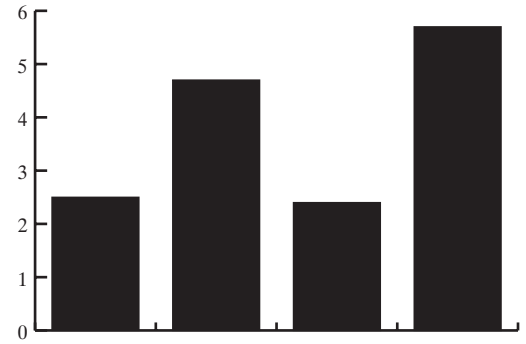
On the basis of the carried out studies on the effect of EMF on the human organism, oxygen metabolism of cells exposed to electromagnetic radiation was found to be the possible indicator of the organism sensitivity to this environmental factor. Some significant biological effects of the systems susceptibility to EMF

Figure 1. **The measurement of free radicals generation with the chemiluminescence method dependent on the exposure time to magnetic field, n=25 (values expressed in thousand impulses per 30 min)**



platelets	0 min	30 min	60 min	90 min
+ EMF	104.876875	155.6300476	103.1443636	121.4622381
SD	33.32015822	55.00504102	18.5001301	19.97156189

Figure 2. **The measurement of malondialdehyde concentration dependent on the exposure time to magnetic field, n=25 (values expressed in nmol/10^9 blood platelets)**



	control	30 min	60 min	90 min
x	2.55	4.63	2.58	5.63
SD	0.29	0.68	0.75	1.01

were recognized [5]. The process of phospholipid peroxidation of cell cytoplasmatic membranes is one of the best described.

The carried out own studies concern the effect of EMF occurring in a car cabin on oxygen metabolism of blood platelets being an important element determining the process of hemostasia in a human organism.

Aim

The aim of the study is to determine to what degree free radicals generation in blood platelets exposed to EM (induction: 0.5 mT, frequency: 1 kHz) occurring in cars affects the process of these morphotic elements cell membranes phospholipid peroxidation.

Material and methods

The suspension of human blood platelets obtained from blood-donation centre from voluntary blood donors was the material for the study. Those donors were examined, contraindications were excluded and laboratory blood tests typical for blood donors were performed. The preparation was transported from the blood bank in a container made of transformer plate which shielded the material against EMF occurring in the car cabin.

The parameters of magnetic waves occurring in the car cabin were reproduced in laboratory conditions. Holmholtz coils generating electromagnetic field which affects blood platelets placed in 8 polyethylene test-tubes (*Fig. 1*) are the main element of the research stand. Each test-tube may contain maximum 2 ml of blood sample. Geometric measurements of Holmholtz coils and their distance are selected in such a way that the magnetic component of the field stimulating the preparation had a homogenous course and was characterised by induction of values occurring in vehicles. The study was carried out according to the demonstrated procedure. The polyethylene test-tube containing blood platelets up to 2 ml of volume was placed in a research stand exposed to the electromagnetic field of induction B 0.5 mT and the frequency 1 kHz for 30, 60 and 90 min to maintain optimal conditions, the ambient temperature of the research stand and the temperature inside the luminometer was kept at 25^0C.

Chemiluminescence measurement was performed in the control and the study sample. The control sample included the platelet suspension with PBS and luminol (indispensable to intensify chemiluminescence) whereas, the study sample electromagnetic field stimulated platelet suspension with PBS and luminol. Chemiluminescence was measured with luminometer Lumicom (HAMILTON) co-operating with IBM computer. Simultaneous sequential measurement was performed for 6 samples.

Malondialdehyde concentration was investigated each time after definite time exposition to EMF (30, 60 90 min) with Placer's method [6]. Follow-up examinations were performed according to the above presented procedure, however with blood platelets not exposed to EMF effect.

The obtained results were statistically analysed with t-Student test for two means at $p \leq 0.05$.

Results

The measurements of free radicals generation performed in accordance with the presented methodology, demonstrated the highest increase of generation after 30 and 90 min exposure to the investigated electromagnetic radiation as compared to the value of the control sample (*Fig. 1*). The highest malondialdehyde concentration was observed also after 30 and 90 min exposure to the investigated electromagnetic radiation as compared to the value of the control sample (*Fig. 2*).

Discussion

Every exogenic factor inducing the increase or decrease of free radicals generation carries with it significant biological consequences [7]. These particles due to high reactivity belong

to destabilizers of biological systems in which they appear [8,9]. Blood platelet is one of the biological systems on which the effect of EMF on free radicals generation is investigated. This cell has a certain specificity in oxygen reactive species generation as compared to other blood morphotic elements.

In blood platelets, there are several sources of oxygen reactive species (ROS) generation. Breathing chain found in mitochondria is one of them. The mentioned cell structures are not found in erythrocytes, which however have in their particle Fe^{2+} atom (of debatable function in ROS generation), while granulocytes defensive function is mainly associated with enzymatic activity of oxidase NADPH (generating anion-radical) [10]. Earlier experiments carried out on blood platelets determined the effect of EMF emitted by cell phones of the frequency 900 and 1800 MHz on blood platelets and exposure of the above mentioned morphotic elements to EMF of power frequency 50 Hz and induction 15 mT. The obtained results demonstrated unambiguously changes in blood platelets oxygen metabolism stimulated with the mentioned EMF radiation [11,12].

At present, the carried out own studies concerning free radicals generation in blood platelets under the effect of EMF of definite parameters, confirm sensitivity of these cells to non-ionizing radiation of definite parameters. The increase of free radicals in blood platelets observed after 30 and 90 min in EMF points to the existence of the dependence between cell oxygen metabolism and the investigated radiation.

Upset balance of intracellular ROS generation consisting in the increase of free radicals compared to the norm was also observed in other studies concerning the effect of non-ionizing radiation on biological systems [13,14]. Keeping the lowest possible level of free radicals generation is important due to their infavourable effects on biological systems. Cell membranes phospholipid peroxidation is one of the best known biological effects of excessive free radicals generation. This free radical chain reaction of oxidation of polyunsaturated fatty acids being included in cytoplasmic membranes phospholipids, was a decissive effect on blood platelets function causing activation of the mentioned blood morphotic elements (adhesion, aggregation). The process of cell membranes lipid peroxidation has been recognized as the effect of the lack of the defensive capability of antioxidative systems and thus remains one of the main pathomechanisms investigated in the case of the determination of cellular changes associated with oxidative stress.

There are scarce studies describing the effect of EMF on the blood platelets antioxidative system. Dependently on the radiation parameters, the increase of malondialdehyde concentration was demonstrated, pointing to the process of cell membranes peroxidation being in progress [15] or to the lack of MDA quantitative changes in comparison to the control samples (such results were often obtained for EMF values used in physiotherpy – stimulation of regenerative processes, among others by the application of magnetic field, proceeds as a result of increased activity of antioxidative defense enzymes and inhibited lipid peroxidation process, what accelerate the regenerative process of the damaged tissues [16].

The observed changes in malondialdehyde concentration prove that there may come to increased peroxidation of arachidonic acid – the component of blood platelets cell membranes phospholipids, under the effect of electromagnetic field emitted by car electronics. The increase of MDA concentration in the investigated time intervals speaks for insufficient adaptation of cell antioxidative mechanisms of blood platelets protecting against oxidation of cell membranes components, with all the above mentioned effects of this process.

Conclusions

1. The observed changes in malondialdehyde concentration prove that there may come to increased peroxidation of arachidonic acid – the component of blood platelets cell membranes phospholipids, under the effect of electromagnetic field emitted by car electronics.
2. The increase of MDA concentration in the investigated time intervals in response to excessive free radicals generation in blood platelets speaks for insufficient adaptation of cell antioxidative mechanisms of the mentioned blood morphotic elements protecting against oxidation of cell membranes components with all biological effects of this process.

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