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The impact of electromagnetic fields with frequency of 50 Hz on metabolic activity of cells *in vitro*

***Abstract**. The article presents the results of research on the influence of the sinusoidal electromagnetic field with a frequency of 50 Hz on the metabolic activity of two cell lines normal BJ and cancer 143b, with different exposure times (0.5 h, 1 h, 2 h and 3 h). It is shown the difference in its effect on cancer and physiological line. In order to analyze the viability there was performed the MTT test.*

Streszczenie**. W artykule zostały zaprezentowane wyniki badań wpływu sinusoidalnego pola elektromagnetycznego o częstotliwości 50 Hz na aktywność metaboliczną dwóch linii komórkowych - normalnej BJ i nowotworowej 143b przy różnych czasach ekspozycji (0.5 h, 1 h, 2 h i 3 h). Wykazano różnice w oddziaływaniu na linię fizjologiczną oraz nowotworową. W celu dokonania analizy żywotności wykonano test MTT.* ***(Oddziaływanie pola elektromagnetycznego o częstotliwości 50 Hz na aktywność metaboliczną komórek w warunkach in vitro)

Keywords: electromagnetic field, the metabolic activity of cells, cancer cells
Słowa kluczowe: pole elektromagnetyczne, aktywność metaboliczna, komórki nowotworowe

Introduction

For many years, scientists are trying to learn how the electromagnetic field affects the living organisms, including human. Many studies have shown that there may be a relationship between exposure to the electromagnetic field of low frequency (EL-EMF) and the illness of cancer in adults and children (eg. leukemia, central nervous system lymphoma) [1, 5]. There are also papers which do not confirm this relationship [3]. There is not known a relationship between radiation dose and its effect.

This paper presents the results of study the effect of EL-EMF with frequency of 50 Hz on the metabolic activity of normal (human fibroblasts; BJ) and cancer (Human osteosarcoma; 143B) cell lines at various exposure times and different values of magnetic induction. Natural emitters of the electromagnetic field in the surrounding environment are the Earth, Sun, Space and phenomena that take place there - electrical discharges, sea tides. In recent decades there has been a huge increase in the number of sources of artificial electromagnetic fields with frequencies not found in the nature. The emitters of such fields are power lines, transformer stations, radar stations, radio navigation equipments, mobile phone base stations and all devices powered by the power network.

The impact of electromagnetic fields on living organisms is usually very complex. It depends on the type of these fields, the size of the intensity and nature of the variability in time, and the properties of the biological structures of the body itself [5]. In mammals, including humans, there is no specialized receptors, which would normally be stimulated by electromagnetic fields informing the central nerve system about its actions. They occur only in the lower evolutionary animals, such as fish and migratory birds and are used inter alia for navigation [4]. Despite the lack of receptivity to electromagnetic fields, it may cause, both in the low and high frequency biological effects among others, synthesis of nucleic acids and proteins [6]. The electromagnetic radiation leads to a sharp increase in the level of reactive oxygen forms, which by oxidation of lipids, proteins, sugars, DNA and RNA cause cells damage [7, 8]. The effects of the electromagnetic field were also observed at the level of the mechanisms regulating cells proliferation as well as programmed cells death - apoptosis, making it a potential therapeutic agent both in cell proliferative diseases such as cancer and in the regeneration treatment [9-12] Our previous studies suggest that EMF can have a significant biological effect on the cells of the pineal gland in a time-dependent exposure to its action [13]. The pineal gland is likely to sense EMFs as light, as a consequence, may decrease the melatonin (MEL) production. Melatonin has been identified as an naturally occurring free-radical scavenger [14] and therefore reduced night time levels can lead to cancer and other serious illnesses.

At the same time there are more and more research results on the positive effect EMF on the neurosecretion activity of centers localized in the limbic system of the human central nervous system, which can be used in the treatment of depression [15]. EMF is used in therapy in physical medicine as a magnet therapy, and the magnetic stimulation.

Materials and methods

The research team of bioelectromagnetism of Institute of Applied Biotechnology and Basic Sciences and the Faculty of Mathematics and Natural Sciences of the University of Rzeszow conducted the study, which aimed at determining the effects of sinusoidal electromagnetic field with values of magnetic induction 2mT, 2.5 mT, 3mT, 4 mT and 6 mT, with frequency of 50 Hz and different times of exposition - 0.5h, 1h, 2h and 3h on metabolic activity of cell lines *in vitro*.

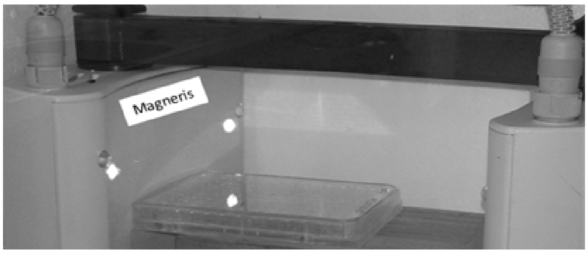

Fig. 1 The cell culture exposed to EMF inside the Magneris system

EMF Exposure System

The source of the electromagnetic field was the Magneris generator (Astar). The distributions of EMF was determined by Astar using: magnetic field meter GM04 (Hirst Magnetic Instruments, UK), Hall effect sensor type A1321 (Allegro MicroSystems), TDS1002B oscilloscope (Tektronix), BM515X digital multimeter (BRYMEN). The device generates a low frequency electromagnetic fields in the range of 2 to 120 Hz, giving the possibility to change the shape for sinusoidal, triangular and rectangular. Electromagnetic field distribution inside the two-piece flat applicator has given the opportunity to conduct study for different values of magnetic induction. The cell culture inside the Magneris system is shown at figure 1.

Cells culture

The human osteosarcoma cell line, 143B, and human fibroblast cell line, BJ, were obtained from the American Tissue Culture Collection. Cell lines were cultured in Roswell Park Memorial Institute (RPMI) 1640 Medium (Lonza), supplemented with 10% foetal bovine serum (FBS) (Gibco) and antibiotics (100 IU/mL penicillin and 100µg/mL streptomycin) (Gibco) as defined by the manufacturer. Cells were grown in 75 cm^2 culture flasks containing 15 ml RPMI 1640. After a few passages cells were seeded in 96-well plate (5 x 10^3 cells per well) and cultured in a humidified atmosphere of 5% CO_2 at 37^{o}C. All studies used cells at 70 to 80 % confluence.

Electromagnetic field exposure

The 96 wells plates were kept inside an EMF generator by the above-specified times. After the exposition there was conducted the MTT assay.

MTT Assay

In order to assess the viability of cells after exposure to electromagnetic fields the MTT assay was performed.

The MTT assay allows measurement of an activity of the energy conversion in mitochondria utilizing the ability of succinate dehydrogenase (an enzyme which is only active in cells with intact metabolism) for the reduction of tetrazolium salt (3- (4,5-dimethyl-2-thiazolyl) -2,5-diphenyl - 2H-tetrazolium bromide, MTT) a yellow-colored to purple colored formazan.

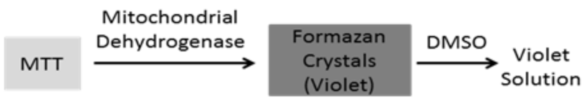

Fig. 2 The diagram of the MTT assay

The amount of produced formazan is proportional to the oxidative activity of cells mitochondria, and thus to the number of metabolically active (viable) cells in a population. In order to calculate the percentage viability it was necessary to measure the absorbance on spectrophotometer. Cells viability was calculated relative to the control, which was taken as 100%, according to the formula (1):

(1) $$V_{(\%)} = \frac{pB}{pK}100$$

where:V (%) - viability percentage,
pB - the average absorbance values for the tested sample,
pK - the average absorbance values for controls.
The diagram of the experiment is shown at figure 2. It shows the stages of the experiment step by step.

Statistical Analysis

The statistical analysis of the results was performed using GraphPad Prism ver. 6.0. All of the results are presents as the means± SD. Differences between control and test samples were assessed with one-way analysis of variance (ANOVA) with Dunnett post hoc test. A p-value of <0.05 was considered as statistically significant.

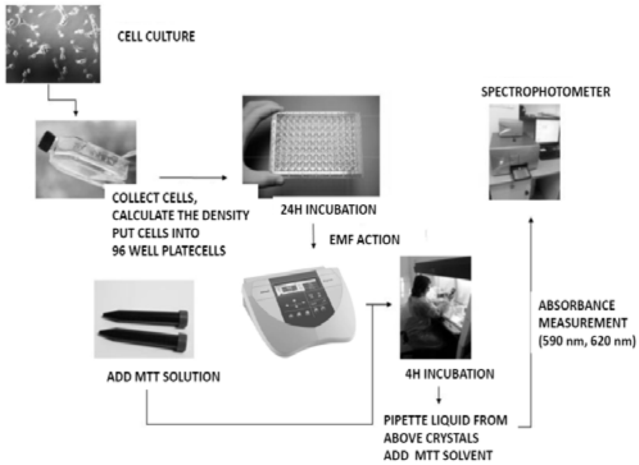

Fig. 3 The diagram of the experiment of the impact of EMF on metabolic activity of cell lines

Experimental results and discussion

There are shown the test results for commercial cell lines 143b (cancer line) and BJ (normal line) after the exposition on EL-EMF. All results were compared with sham-exposed control cells. The Fig. 4 shows the results for 143b cell line and Fig. 5 for BJ cell line for different times of exposition and different values of magnetic induction.

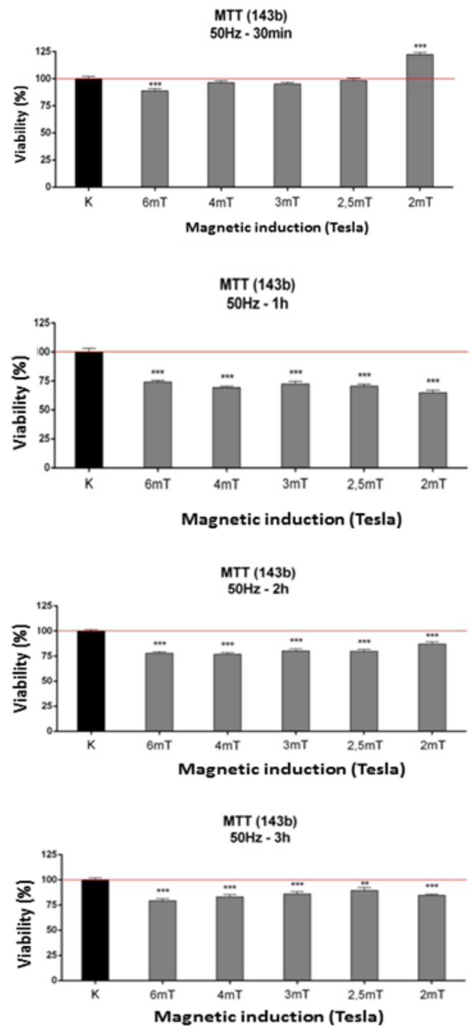

Fig. 4 The viability (%) of 143b cell line treated with 0.5, 1, 2, and 3 hours of an electromagnetic field with a frequency of 50 Hz and magnetic induction in the range 2-6mT. The results are presented as the mean ± SEM; ** P≤0.01; *** P≤0.001.

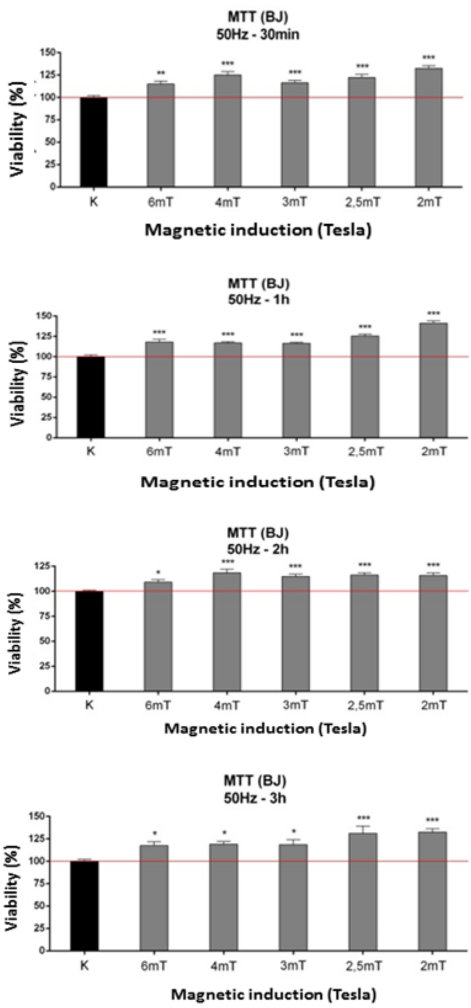

Fig. 5 The viability (%) of BJ cell line treated with 0.5, 1, 2, and 3 hours of an electromagnetic field with a frequency of 50 Hz and magnetic induction in the range 2-6mT. The results are presented as the mean ± SEM; ** P≤0.01; *** P≤0.001.

The results of the study showed a significant effect of the sinusoidal electromagnetic field with a frequency of 50 Hz on the viability of analyzed two cell lines – cancer and normal. There was a decrease in cell viability of 143b line, however, it depends on the exposure time (Fig. 4). Significantly lower viability with regard to control characterized cells subjected to at least 1-hour exposure to electromagnetic fields in each of the analyzed values of magnetic induction. The result of the half-hour exposure of cells to electromagnetic fields was a significant decrease in cells viability for magnetic induction 6mT with a simultaneous significant increase in viability in conditions of 2mT magnetic induction (Fig. 4). Opposite effect of the influence of electromagnetic fields on cells viability was observed in BJ cells line. After each of the analyzed time of exposure to electromagnetic fields there was observed a significant rise in the viability of cells compared to the control (Fig. 5). The mechanisms by which EMF affects biological processes are not well established. Some research proposed non-specific processes such as the generation of heat, formation of free-radicals, and promotion of DNA damage [16-18]. Exposure of cells to 20-60 Hz EMF patterns has been shown to affects on cAMP levels, MAP kinase activation, Ca^{2+}-calmodulin kinase activation, or Ca^{2+} channels [19-22]. However, none of these mechanisms does explain the differences in response to the EMF between cancer and normal cells. Research conducted by Buckner et al (2015) were showed that exposure of malignant cells to EMF (for > 15 min) promoted Ca^{2+} influx and inhibition of cell proliferation. Non-malignant cells did not show any EMF-dependent changes in Ca^{2+} influx or cell growth [23]. These observations are consistent with the idea that exposure to specific EMF patterns could provide a potential anti-cancer therapy.

Summary

Studies on the effects of electromagnetic fields on living organisms are an important element of the biophysical studies. These are interdisciplinary research, combining inseparable biology, medicine and issues of electrodynamics and electrical metrology. Scientists research have suggested the influence of electromagnetic field exposure and the occurrence of childhood leukemia, brain tumors, genotoxic action, or the degradation of the immune system. People are exposed to EMF every day. Consequences of EMF effects on living organisms are not fully understood and depends on the type of cells which are affected, the exposure time, the type and intensity of field.

In our study we examined whether the time of exposition on sinusoidal EMF with frequency of 50 Hz, and the value of magnetic induction (from 2 to 6 mT) affect the viability of two cell lines - BJ (normal line) and 143b (cancer line). The obtained results showed that the effect on the tested cell lines is different and statistically significant depends on the kind of line - cancer compared to the normal, and also depends on the value of the field and the exposure time.

After the exposition on the electromagnetic field there was observed a significantly different viability decrease in cancer line, but in normal cells the increase of viability. These results suggest that treatment with the electromagnetic field may be applicable in oncology and rehabilitation.

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Declaration of interest

The authors declare no conflict of interest.

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