Anna KOZIOROWSKA [1,3], Katarzyna KOZIOŁ[2], Sebastian GNIADY [3], Maria ROMEROWICZ-MISIELAK[3]

[1] Uniwersytet Rzeszowski, Wydział Matematyczno-Przyrodniczy, Katedra Inżynierii Komputerowej
[2] Uniwersytet Rzeszowski, Wydział Biotechnologii, Katedra Fizjologii i Rozrodu Zwierząt
[3] Uniwersytet Rzeszowski, Wydział Biotechnologii, Laboratorium Bioelektromagnetyzmu

doi:10.15199/48.2018.06.25

An electromagnetic field with a frequency of 50 Hz and a magnetic induction of 2.5 mT affects spermatogonia mouse cells (GC-1spg line)

Abstract. *The article presents the results of studies on the influence of electromagnetic field with a frequency of 50 Hz and a magnetic induction of 2.5 mT on morphology, viability and proliferation, and the changes in the biochemical profile of cells of the GC-1 spg (mouse spermatogonia B) in vitro. Giemsa staining was used to evaluate the morphology of the cells. Cell viability was assessed by trypan blue staining. The degree of cell proliferation was determined by the cell density in the culture. On the other hand, changes in the biochemical profile of cells were determined using FTIR spectroscopy in infrared. Based on the results of the study, the effect of EMF on cell viability was determined according to the exposure time.*

Streszczenie. *W artykule przedstawiono wyniki badań nad wpływem pola elektromagnetycznego o częstotliwości 50 Hz i indukcji magnetycznej 2.5 mT na morfologię, żywotność i proliferację, a także zmianę profilu biochemicznego komórek linii GC-1 spg (mysie spermatogonia typu B) w warunkach in vitro. Metodą oceny morfologii komórek było barwienie metodą Giemsy. Przeżywalność komórek oceniano za pomocą barwienia błękitem trypanu. Stopień proliferacji komórek określano na podstawie gęstości komórek w hodowli. Natomiast, zmiany w profilu biochemicznym komórek ustalono wykorzystując spektroskopię FTIR w podczerwieni. Na podstawie przeprowadzonych badań wykazano wpływ pola elektromagnetycznego na przeżywalność komórek w zależności od czasu ekspozycji.* ***(Pole elektromagnetyczne o częstotliwości 50 Hz i indukcji magnetycznej 2.5 mT wpływa na komórki linii GC-1spg warunkach in vitro)***

Keywords: electromagnetic field, proliferation, viability, biochemical profile, GC-1 spg cells
Słowa kluczowe: pole elektromagnetyczne, proliferacja, przeżywalność, profil biochemiczny, komórki linii GC-1 spg

Introduction

In the era of technology and telecommunication development, there are more and more sources of electromagnetic field with frequencies previously not found in nature.

The electromagnetic field is a system of two interrelated fields: electric and magnetic, and it is possible at each point of it to determine the vectors of electric field strength E and intensity of the magnetic field H [Gryz et al. 2011]. Electrical component of the electromagnetic field means the interaction between electrically charged particles or bodies moving in any way in relation to the inertial reference system [Różycki 2011]. The vector of an electric field intensity describing an electric field, which unit in the SI system is Newton on a coulomb can be defined by the formula:

$$\mathrm{E} = \frac{\mathrm{F}}{\mathrm{q}} \tag{1}$$

where: F - force that the electric field exerts in a given location per unit point charge,
q - value of this charge.

The magnetic component of the electromagnetic field determined in amperes per meter is produced by the change of electric field over time by the system of moving loads. The magnetic field intensity vector H can be expressed as:

$$\oint \mathrm{H} \times \mathrm{dI} = \mathrm{I} \tag{2}$$

where: H - intensity of the magnetic field, I - current flowing through any surface extending over the closed contour.

The electromagnetic field can affect physical objects, including living organisms. The effect of its influence depends on many factors, and the most important are:
1) field intensity;
2) the distance of the object from the field emission source;
3) frequency of radiated energy (Halliday et al., 2001).

Numerous scientific studies have shown that the male reproductive system is one of the most sensitive organs to electromagnetic radiation. Exposure to electromagnetic field of extremely low frequency (0-300 Hz) (ELF-EMF), both in humans and animals, can lead to decreased sperm quality by reducing motility, viability and sperm morphological changes and an increase in the level of oxidative stress (Lee et al., 2004; Kim et al., 2009; Barnabo et al., 2007; Barnabo et al., 2010; Iorio et al., 2007; Luo et al., 2012; Esposito et al., 2013; Roychoudhury et al., 2009). Differences in cell proliferation and viability are directly related to electromagnetic field frequency and exposure time (Kiray et al., 2013; Bedir et al., 2015). Despite various methodological approaches to explaining the mechanism of action of ELF-EMF on biological systems, it still remains unclear. One of the reasons of lowering the rate of proliferation of cells exposed to electromagnetic fields may be the induction of programmed cell death (apoptosis). In mice, an electromagnetic field with a frequency of 60 Hz and magnetic induction 0.5 mT induces spermatogonia apoptosis and DNA damage, but does not significantly affect the viability of germline nuclei (Lee et al., 2004). The mechanism of induction of apoptosis in the germ cells as a result of ELF-EMF has not been known until now. The cells of the male reproductive system due to its structure and function are particularly sensitive to DNA damaging agents. An exposure of the cells of mouse spermatocytes GC-2 to an electromagnetic field with a frequency of 50Hz and magnetic induction of 3mT resulted in a significant increase in the amount of DNA strand breaks (Duan et al., 2015). There is also the evidence that an exposure to electromagnetic fields of extremely low frequency (50Hz, 3mT) in fetal life causes abnormal development of the testes and results in abnormal spermatogenesis in adult life (Hamdi et al., 2011). In humans, exposure to electromagnetic fields of extremely low frequency, in vitro, may in turn lead to an increase in sperm motility in a manner dependent on the exposure time and

characteristics of the field (Iorio et al., 2007; Wdowiak and Mazurek, 2017). The above data indicate the different effects of electromagnetic field on reproductive cells, which depends on the species, the type of cells, and the electromagnetic field and exposure time.

The aim of this study was to investigate *in vitro* the effect of the electromagnetic field with a frequency of 50 Hz and a magnetic induction of 2.5 mT on the morphology, viability and proliferation, as well as changes of the biochemical profile of mouse spermatogonial cells of the type B (GC-1 cell line spg).

Materials and methods

Cells culture

GC-1 spg cells, a mouse-derived spermatogonial cell line (ATCC), were cultured at 37^{o}C in DMEM medium supplemented with 10% fetal bovine serum and antibiotic and antimycotic mix solution (100 U/ml penicillin, 0.1 mg.ml streptomycin and 0.25 µg/ml amphotericin B) (Sigma) in humidified atmosphere in the presence of 5% CO_2. At 80% confluence, the cells were subcultured with 0.05% trypsin (Gibco) for further experiments.

EMF Exposure System

The source of the electromagnetic field was the Magneris generator with an innovative two-piece flat applicator (Astar). The device generates a low frequency electromagnetic fields in the range of 2 to 120 Hz and gives the possibility to change the shape for sinusoidal, triangular and rectangular. 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).

Exposure Procedure

GC-1 cells were plated onto T25 flask at a density of 1 x 10^5 cells/1ml. The cells were exposed to a sinusoidal electromagnetic field of 50 Hz and a magnetic induction of 2.5 mT or sham conditions for 30 min/day for 10 days.

Viability and Cells Proliferation Assay

The analysis of cells viability and proliferation was performed using trypan blue exclusion tests (0.4 % in phosphate-buffered saline). Cell number was measured each day using hemocytometr. Blue stained cells were considered nonviable. Percent inhibition of cell proliferation was calculated as the difference in cell number between sham and EMF-treated cultures.

Cells morphology observation

Cells morphology was observed under inverted microscope every day. Giemsa staining was carries out after 0, 2, 6, and 10 days exposure. Cells were rinsed by Phosphate-buffered saline (PBS) twice after medium were removed in T25 flask, then fixed with 5 ml 75% methanol for 15 minutes and stained with Giemsa for 20 min at Room Temperature. Afterward, cells were washed and the cell morphology were observed under inverted microscope (Olympus BX43).

Infrared Spectroscopy

Spectral data of GC-1 cells, exposed or not exposed, were collected using Fourier Transform Infrared (FTIR) spectrometr (Nicolet iZ 10 module, Thermo Fisher Scientific Inc., United States) equipped with a deuterated triglycine sulfate (DTGS) detector, KBr beam splitter, and a diamond single bounce Attenuated total reflectance (ATR) accessory. Collection, pre-processing, and analysis of spectral data were performed by means of OMNIC software (v. 9.0, Thermo Fisher Scientific Inc.). To perform the FTIR analysis, the suspension of EMF-treated cells and control cells was centrifuged for 5 min. at 400xg. The culture medium was dropped and the cells were rinsed with 3 ml of PBS. Then centrifuged for 5 min. at 400xg. The rinse was repeated twice. After the last wash PBS was removed and the cells were frozen at -80 ° C for 24 h. Frozen cells were lyophilized for 24 h.

Results

Effects of ELF-EMF exposure on cells morphology

As a result of exposure to the electromagnetic field of 50 Hz and magnetic induction of 2.5 mT the cells morphological changes such as irregular nuclei, nuclear-cytoplasmic change, thickened cell membrane, abnormal coloration, or presence of apoptotic cells were observed (Fig. 1).

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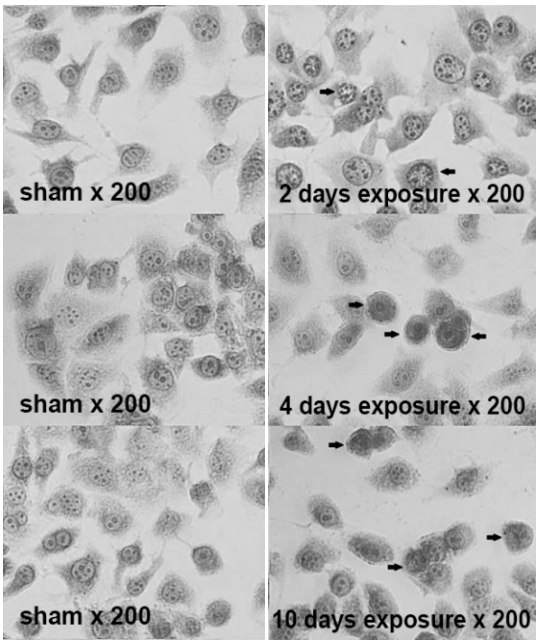

Fot. 1. The cell morphology and nuclear morphology of GC-1 spg cells after 2, 6 and 10 days ELF-EMF exposure.

Effects of ELF-EMF exposure on cells viability

Trypan blue exclusion tests showed that the viability of GC-1 spg cells decreased after 10 days exposure to ELF-EMF compared with sham group (Fig. 1.).

Effects of ELF-EMF on cell proliferation

The results of proliferation rate of GC-1 spg cells are shown in Figure 2. After 6 days, a half-hour exposure per day to an electromagnetic field with a frequency of 50 Hz and a magnetic induction of 2.5 mT there was a decrease in cell proliferation rate compared to the control group. This effect lasted until the 10th day, until the end of the experiment (Figure 2).

Changes in the biochemical profile of GC-1 cells treated with EMF

Spectra of lyophilized GC-1 cells treated with EMF at 50 Hz and magnetic induction of 2.5 mT for 30 min. daily for 10 days and cells not treated with EMF are shown in Figure 3. The greatest differences in absorbance were observed for wave numbers 1158 cm^{-1} and 860 cm^{-1}. The cells exposed to an electromagnetic field showed increased absorbance in these regions, suggesting an increase in the level of phosphate groups, and aromatic compounds in the treated cells.

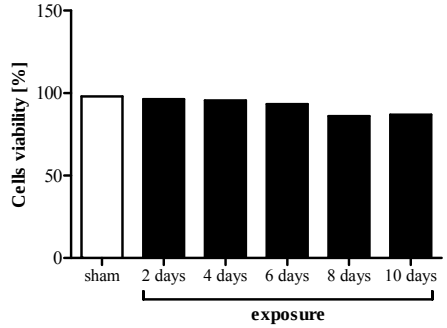

Fig. 1. The cell viability of GC-1 spg cells after 2-10 days ELF-EMF exposure vs. sham group

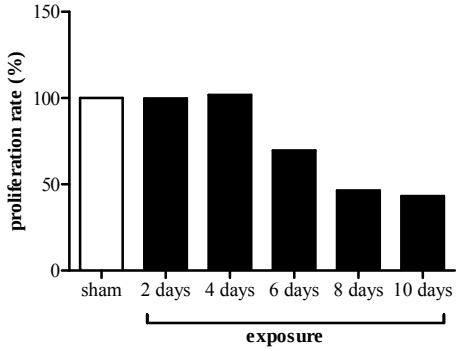

Fig. 2 The proliferation rate (%) of GC-1 spg cells after 2-10 days ELF-EMF exposure vs sham group

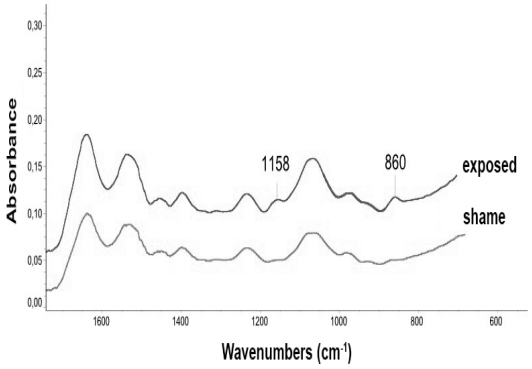

Fig. 3. ART-FTIR spectra of 50-Hz EMF-induced GC-1 cells and a magnetic induction of 2.5 mT for 10 days vs. shame.

Discussion

The technological revolution that began in the twentieth century has resulted in an increase in the number of devices generating electromagnetic fields of different frequencies with which we deal in everyday life. Studies on the effects of EMF on biological systems have yielded contradictory results (Kasprzyk and Butlewski, 2013). There can not be unequivocally confirmed neutral, negative or positive effect of electromagnetic field. The biological effects of EMF depend on the species and type of cells on which it operates, the time of exposure and electromagnetic field parameters (Wdowiak and Mazurek, 2016).

In the present study we investigated the effects of EMF with a frequency of 50 Hz and a magnetic induction of 2.5 mT, *in vitro,* for proliferation, viability, morphology and the biochemical profile of GC-1 spg cell line.

The presented results indicate that exposition to ELF-EMF inhibits the rate of proliferation of GC-1 spg cells. Reducing of cell division frequency was observed after six days of daily half-hour exposure to EMF (50 Hz, 2.5 mT). It suggests that prolonged exposure to ELF-EMF may affect the proliferation and differentiation of spermatogonia. The studies conducted on 7-week-old mice showed that 8-week electromagnetic field exposure at 60 Hz and 0.1 mT or 0.5 mT induced growth of amount of germinal cells in the late apoptotic phase (Lee et al., 2004).

The performed study did not show increased cell apoptosis in the test sample compared to control (Liu et al., 2015). The morphological evaluation of cells exposed for 10 days to EMF (30 min, 50 Hz, 2.5 mT) showed the presence of irregular cell nuclei, bold cell membrane, altered nuclear-cytoplasmic relation as well as apoptotic cells.

The analysis of changes in the biochemical profile of GC-1 cells treated with EMF showed an increase in the levels of phosphate groups and aromatic compounds, which demonstrates the influence of EMF on cell metabolic activity associated with the activation of phosphorylation and the formation of organic aromatic compounds, as well as ATP.

The mechanism of interaction of EMF on cells of the reproductive system is still not completely understood, and its deeper understanding at the molecular level requires further study and analysis.

Acknowledgements

We would like to thank Prof. Jacek Żebrowski (Department of Plants Physiology, Faculty of Biotechnology, University of Rzeszow) for performing FTIR analysis.

The study was carried out with the use of equipment granted by the project "Centre of Applied Biotechnology and Basic Sciences" supported by the Operational Programme "Development of Eastern Poland 2007-2013". No. POPW.01.03.00-18-018/09,

Part of the research was carried out with funding from the municipal office of Kolbuszowa, Poland.

Author contributions

A. K., K. K., M. R-M. conceived and designed the experiments and wrote the paper.

S. G. performed the experiments.

A. K., K. K., M. R-M. analyzed the data.

***Authors**:*

PhD Eng. Anna Koziorowska, University of Rzeszow, Faculty of Mathematics and Natural Sciences, Institute of Biotechnology, Laboratory of Bioelectromagnetism;

E-mail: akozioro@ur.edu.pl

PhD Katarzyna Kozioł, University of Rzeszow, Institute of Biotechnology, Department of Physiology and Reproduction of Animals;

E-mail: katko@ur.edu.pl

Eng. Sebastian Gniady, student, University of Rzeszow, Institute of Biotechnology; *Laboratory of Bioelectromagnetism;*

PhD Maria Romerowicz-Misielak, University of Rzeszow, Institute of Biotechnology, Laboratory of Bioelectromagnetism.

E-mail: m_romer@interia.pl

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