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doi:10.15199/48.2019.05.02

Electromagnetic field as an environmental factor affecting MCF-7 cell line *in vitro*

Abstract. *The purpose of the experiment was to obtain a response whether the electromagnetic field at the frequency of 50 Hz and magnetic induction of 2.5 mT affect the viability, proliferation and ability to migrate of MCF-7 cell line(the human breast adenocarcinoma cells) The viability of cells was assessed by trypan blue staining. The degree of cells proliferation was determined based on the cells density in culture, and the cells' ability to migrate was determined using plate clone assay and scratch adhesion test..The obtained results indicate the different effect of the EMF with the frequency of 50 Hz and magnetic induction of 2.5 mT on the viability, proliferation and the ability to migrate of the MCF-7 cell line in a manner depending on the time of exposure during the day (30 min, 10 min) as well as number of EMF exposure days (3 days, 6 days, 9 days).*

Streszczenie*. Celem doświadczenia było uzyskanie odpowiedzi czy pole elektromagnetyczne o częstotliwości 50 Hz i indukcji magnetycznej 2,5 mT wpływa na żywotność, proliferację oraz zdolność do migracji komórek linii MCF-7 (komórki ludzkiego gruczolaka piersi). Przeżywalność komórek oceniano za pomocą barwienia błękitem trypanu. Stopień proliferacji komórek określano na podstawie gęstości komórek w hodowli a, zdolność komórek do migracji ustalano wykorzystując plate clone assay oraz scratch adhesion test. Otrzymane wyniki wskazują na różny wpływ pola elektromagnetycznego (50 Hz, 2.5 mT) na żywotność, proliferację i zdolność do migracji linii komórkowej MCF-7 w sposób zależny od czasu ekspozycji w ciągu dnia (30 minut, 10 minut), jak również od liczby dni ekspozycji (3 dni, 6 dni, 9 dni).* ***(Pole elektromagnetyczne jako czynnik środowiskowy oddziałujący na komórki linii MCF-7 in vitro)***

Keywords: electromagnetic field, proliferation, viability, biochemical profile, MCF-7 cells
Słowa kluczowe: pole elektromagnetyczne, proliferacja, przeżywalność, profil biochemiczny, komórki linii MCF-7

Introduction

The electromagnetic field (EMF) is defined as the energy state of the space that surrounds the electric charges in motion. The propagation of this energy takes place through electromagnetic radiation [8], which is characterized by the orderly and mutual interaction of magnetic and electric fields. The occurrence of electric charges with opposite signs results in the creation of an electric field [5], which characteristic magnitude is the intensity expressed in the SI system in newtons per coulombs. However, the intensity of the magnetic field is expressed in amperes per meter and is generated by the movement of these charges - the flow of electric current [5]. One of the main parameters describing the electromagnetic field is the frequency f, expressed in the SI system in hertz [Hz] and determining the variability of EMF over time [8]. There is a natural electromagnetic field in the environment, which is emitted by the Sun and Earth and artificial, resulting from the construction of infrastructure emitting fields of unprecedented frequencies - base stations of mobile telephony, high voltage transmission lines, transformer or radar stations, or radio navigation devices [6]. The rapid development of technology has led to an uncontrolled increase in the number of field emitters that overlap each other to create electromagnetic noise. All everyday equipment, powered from the industrial network, generates a 50 Hz field, while EMF in the radio and microwave range (100 kHz - 300 GHz) is produced, among others via TV and radio transmitters, cell phones or microwave ovens.

The EMF is used in both diagnostics and therapy. The field used in medicine achieves the value of magnetic induction from picotesla to a few tesla, and the frequencies used assume values from 0 to gigahertz [9] Exposure of living organisms to EMF can cause various biological effects depending on the intensity, frequency, dose and time of exposure to EMF. It has been demonstrated that electromagnetic field (EMF) in some short-term exposure conditions may affect the biological properties of the cell, such as proliferation [7; 10, 11] and apoptosis [1], which proves that the electromagnetic field can be a potential tool for the manipulation of behavior and cell viability thus opening a new field of research important for future clinical use

Cancer is the first cause of death in a global scale and the second in Europe. The most common cancer diagnosed in women is a breast cancer. The mammary adenocarcinoma (adenocarcinoma mammae) is formed by the abnormal mechanisms of cell proliferation of glandular epithelium. The tumor is formed by numerous lethal, cumulative changes in the genetic material of somatic cell, leading to disorders of control of cell proliferation, differentiation and growth. Risk factors for breast cancer can be divided into some groups. One can be hormonal. The use of birth control pills, endogenous estrogens and progesterone and synthetic hormone replacement therapy may increase the risk of breast cancer. The second group can be emotional factors: stress increases cortisol secretion by adrenal gland, inhibit immune function, promotes tumor growth. There can be distinguished a group of environmental factors, as: exposure to carcinogens: radiation, pesticides, cigarette smoking, alcohol consumption and exposure to electromagnetic fields [3]. Despite significant progress in the field of diagnostics, development of modern methods of treatment, health promotion and the increasing role of prevention, neoplastic diseases are still a big medical and social problem. Contemporary oncology does not have a fully effective and at the same time minimally invasive anti-cancer therapy. It prompted us to carry out the research shown in this paper.

The aim of the study was to evaluate the biological effects of low frequency electromagnetic field in the human breast adenocarcinoma cells (MCF-7). We checked the viability, evaluate of cell morphology, the proliferation and the ability to migrate of MCF-7 cells under the influence of EMF with the frequency of 50 Hz and magnetic induction of 2.5 mT.

Materials and methods

MCF-7 cells culture

The human breast adenocarcinoma cells (MCF-7), were cultured in 25cm^2 culture vessels, at 37°C in DMEM medium supplemented with 10% fetal bovine serum and antibiotic

and antimycotic mix solution in humidified atmosphere in the presence of 5% CO_2. The total number of cells and survival was estimated by trypan blue exclusion.The culture was observed and photographed using a Zeiss Axiovert 40 CFL inverted microscope coupled to a CCD monochrome camera and a computer with AxioVs 40 V 4.8.1.0 software.

EMF Exposure System

The electromagnetic field was generated by the Magneris generator with two-piece flat applicator (Astar, Poland). The system was described in our previous papers [6,7].

Exposure Procedure

To identify the effect of electromagnetic field (EMF) exposure on cell behaviour, cells of the breast cancer cell line MCF7 were exposed or sham-exposed to 50 Hz EMF at 2.5 mT for three days (30 min/day) or nine days (10 min/day). The diagram of the experiment is shown in Fig. 1:

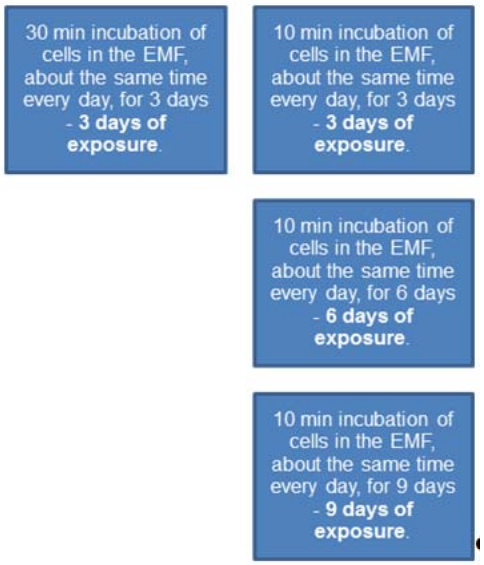

Fig. 1. The scheme of the EMF exposure on MCF-7 cells

After at least 18 hours after passage, the cells were subjected to an EMF. After each EMF interaction into cells, the culture medium was changed to fresh. The control cells for particular field times were cultured identically to the treated cells except the exposure to EMF.

Morphology

Cell morphology was observed under inverted microscope every day. Giemsa staining was carried out after every 3 days of exposure. Cells were rinsed by PBS twice after medium removed, then fixed with 75% methanol for 15 minutes and staining with Giemsa for 20 min. Afterwards, cells were washed and the cell morphology were observed and photographed using a Zeiss Axiovert 40 CFL inverted microscope coupled to a CCD monochrome camera and a computer with AxioVs 40 V 4.8.1.0 software.

Plate clone assay

The MCF-7 cells treated by EMF and the control group were seeded in a 24-well plate at 300 cells per well during each passage and incubated at 37° C under 5% of CO_2 for 7 days. After this time, the DMEM culture medium was removed and the cells were washed twice with 1 ml of PBS. The colonies were then fixed 15 min with 75% methanol and stained with Giemsa for 20 minutes. After dissolution of Giemsa staining cells were rinsed again with PBS. Stained cells were photographed using an Olympus BX43 light microscope coupled to an Olympus UC 30 camera and a computer with the CellSens Dimension image analysis programm.

Scratch adhesion test

The cells of the MCF-7 line treated with EMF and the control group during each passage were seeded on a 24-well plate in the amount of 10,000. cells per well and incubated at 5% of CO_2 at 37° C until the monolayer was obtained. Next, using a sterile 200 µl tip, a scratch was made to create wounds. after rinsing with PBS three times, the wells were refilled by DMEM (1 ml). The wounds were photographed at the beginning (t=0 h) and every 24h until the wounds were completely overgrown, using a reverse microscope (Zeiss Axiovert 40 CFL) coupled with a monochrome CCD camera and AxioVs 40 V 4.8.1.0 software. A program for automatic microscopic image analysis - TScratch [4] was used to calculate the wound size.

Results

Evaluation of cell viability

The determination of the viability of cells exposed to an EMF at 50 Hz and a magnetic induction of 2.5 mT, relative to the control group was made using trypan blue exclusion test.

Figure 2 shows the experiment results of 30 min impact of EMF on MCF-7 cell line. The increase in viability of cells is demonstrated: the cells under the EMF conditions - 97.78%, the control group 92.86%.

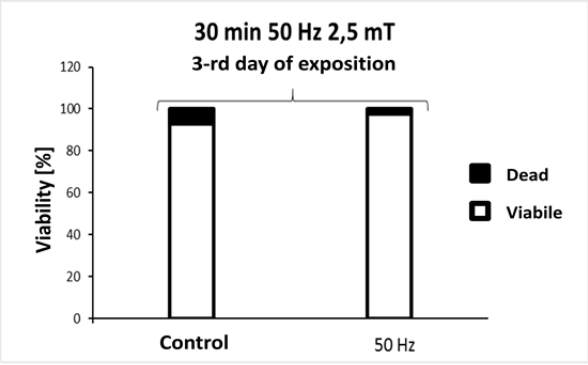

Fig. 2 Percentage of dead and viable cells in culture of MCF-7 cells subjected to an EMF with a frequency of 50 Hz and a magnetic induction of 2.5 mT, day 3, 10 minute of exposure by each day compared to the control culture.

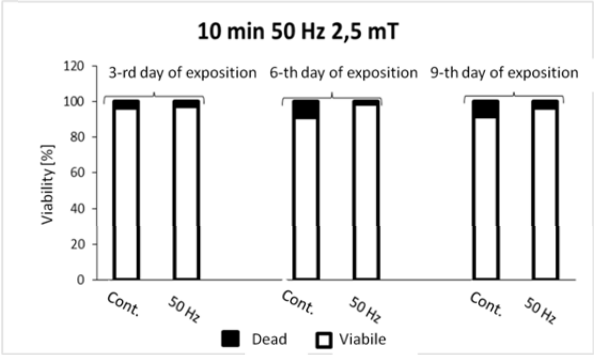

Fig. 3 Percentage of viable and dead cells in MCF-7 cultures treated with 50 Hz EMF and 2.5 mT magnetic induction after 3, 6 and 9 days of 10-minute exposure each day compared to the control culture

In the case of a 10-minute incubation after 3 days of EMF exposure, the cells viability was 97.18% and it was slightly higher than in the group of the untreated cells - 96.52%. There was also a significant increase in the viability of MCF-7 cells influenced by EMF for 10-minutes by 6 days (98.72%) in relation to control (91.15%). However,

after a further 3 days of incubation in EMF, the cells viability decreased to 96.50%, and the cells not treated with EMF were similar (91.54%). There was also a decrease in viability of control cells after 6 (91.15%) and 9 (91.54%) exposure days, compared to a 3-day incubation (96.52%) (Figure 3).

Evaluation of cell morphology

Morphological evaluation of cells treated with EMF 30 min for three days and EMF non-treated cells revealed distinctive features of cellular atypy, characteristic of tumor cells - cell enlargement, irregular cell nucleus contour, irregular thickening of the cell membrane, and changes in cell shape. There are also visible cells with numerous or single nuclei, as well as figures of mitotic divisions, which are defined as normal (Photo 1).

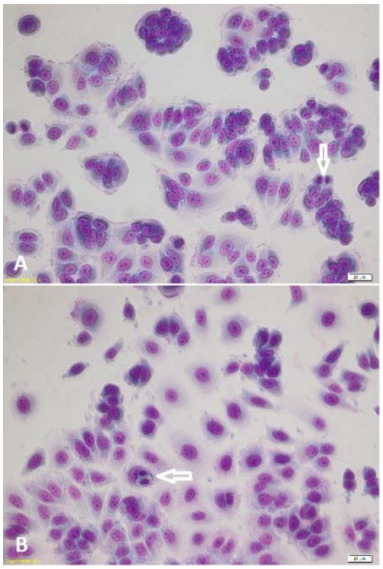

Photo. 1 Morphology of cells after the 3rd day of the 30-minute exposure to EMF with a frequency of 50 Hz and a magnetic induction of 2.5 mT (B) compared to controls (A); the arrow is marked with mitotic figures; 20x magnification

Evaluation of the ability of cells to migrate in vitro using a scratch adhesion test

MCF-7 cells exposed for 30 minutes for 3 days to the EMF were characterized by faster migration to the cell-free area compared to untreated cells. After 72 hours of cells incubation under 5% CO_2 at 37° C, the wound was completely closed in the culture and control (Fig. 4, Photo. 2).

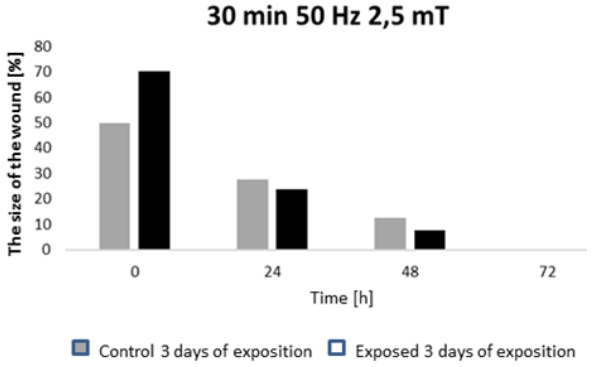

Fig. 4 Wound overgrowth rate of MCF-7 line cells after 3 days of a 30-minute EMF exposure each day with 50 Hz and 2.5 mT magnetic induction, compared to the control, expressed in percent

There was demonstrated a possible negative effect of a 10-minute exposure to EMF with a frequency of 50 Hz and 2.5 mT magnetic induction on the ability of MCF-7 cells to migrate in culture. After 3 days of EMF treatment, the cells were characterized by faster migration to the cell-free area compared to the control. However, after 6 and 9 days of cell exposure to EMF, their ability to overgrow the wounds compared with cells not treated with EMF decreased.

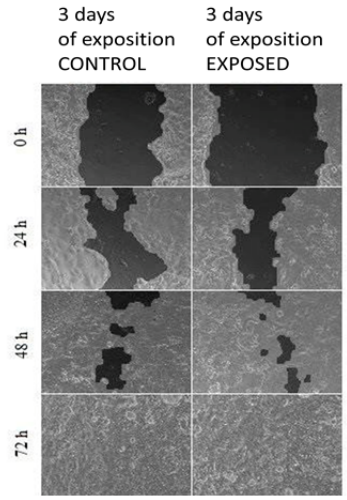

Photo 2 Migration of MCF-7 line cells after 3 days of a 30-minute EMF exposure each day with 50 Hz and 2.5 mT magnetic induction, compared to the control in the scratch adhesion test.

After 6 days of incubation of the cells under the EMF, the slowest overgrowing of the wound was demonstrated, after 72 hours the wound area was approximately 5.6%. There was also showed a faster wound overgrown by cells untreated EMF as compared to exposed ones (6 and 9 days), after 9 days of incubation, the complete closure of the wound occurred until 48 hours (Fig. 5; Photo 3).

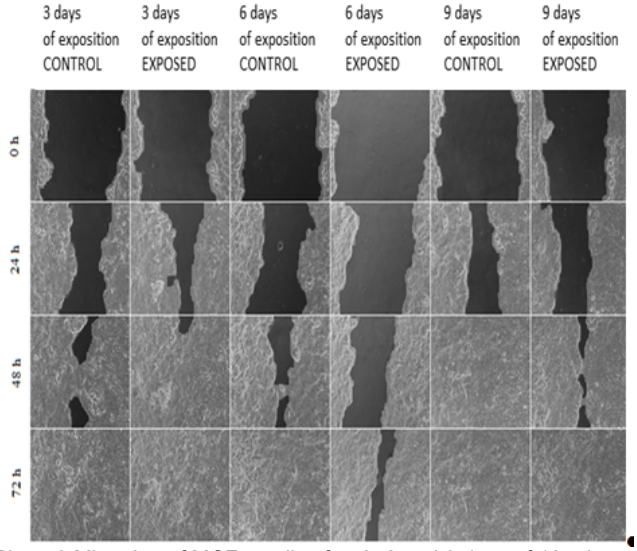

Photo 3 Migration of MCF-7 cells after 3, 6 and 9 days of 10-minute EMF exposure each day at 50 Hz and 2.5 mT magnetic induction compared to controls in the scratch adhesion test.

Discussion

Malignant breast cancer according to statistics is the most common cancer diagnosed in women. It is reported that breast cancer is responsible for 25-30% of all cancers in women [12]. In the last decade, there has been a rapid increase in the incidence in Poland, mainly in women between 50 and 69 years of age. In 2012, there were recorded approx. 17000 cases of illness and 5.5 thousand of deaths due to malignant breast cancer [13].

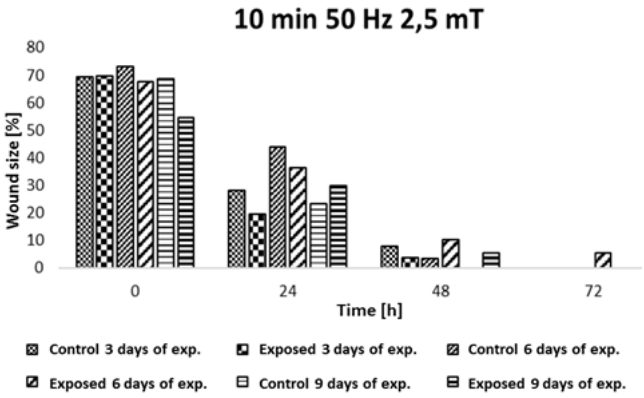

Fig. 5 The rate of the wound overgrowing as a percentage of the cell line MCF-7 at 3, 6 and 9 days on a 10-minute EMF exposure each day - frequency of 50 Hz and a magnetic induction of 2.5 mT, compared to control

The aim of this study was to examine whether EMF at 50 Hz and 2.5 mT magnetic induction affect the viability, proliferation and ability to migrate of MCF-7 cells subjected to 30-minute exposure for 3 days and 10-minute exposure to 3, 6 and 9 days. Cells vaiability was assessed by trypan blue staining and the cell's ability to migrate was determined using a scratch adhesion test.

There were shown a diverse effects of EMF with a frequency of 50 Hz and 2.5 mT magnetic field induction on viability and proliferation as well as the ability to migrate of MCF-7 cell lines depending on the exposure time per day (30 min, 10 min) as well as the number of exposure days (3 days, 6 days, 9 days). In the case of incubation of the cells by 30-minute per day for 3 days, in the conditions of 50 Hz EMF and 2.5 mT magnetic induction, increased viability and faster migration to the cell-free area was shown, as compared to non-EMF treated cells. However, in the case of 10-minute exposure of cells to the EMF, an increase in viability and cell colony formation was observed after 6 days in relation to the 3-day exposure, while after 9 days of exposure a decrease in viability was demonstrated comparing with a 6-day EMF interaction. The EMF-treated cells were characterized by a higher viability compared to the control. A slower migration of cells exposed to EMF by 10 minutes to the cell-free area as compared to the control was also observed. The obtained results indicate the differential effect of the 50 Hz EMF and 2.5 mT magnetic induction on the viability, proliferation and the ability to migrate of MCF-7 cells in a manner depending on the time of exposure during the day (30 min, 10 min) as well as number of days of EMF exposure (3, 6, 9 days).

To unambiguously determine whether in the future it will be possible to apply an EMF in an innovative therapy that will allow the selective removal of cancer cells from a heterogeneous population of malignant and normal cells, further *in vitro* and *in vivo* research are needed at the molecular level.

Acknowledgements

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.,M. R-M. conceived and designed the experiments and wrote the paper.
K. K, E. H. performed the experiments.
A. K., K. K., M. R-M. analyzed the data.

Declaration of interest
The authors declare no conflict of interest.

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