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Examination of constant DC magnetic field influence on apoptosis of human leukemia cell line HL60

***Abstract**. This article provides the outcomes of in vitro tests which were aimed at finding the impact of constant magnetic field on apoptosis of human leukemia cell line HL60. The tests were carried out with the use of a test bed that was designed and built in the Laboratory for Research of EMF Hazards and Bioelectromagnetism of Wroclaw University of Technology. The results of medical tests were obtained in cooperation with the medical staff of the Chair & Clinics of Haematology, Blood Cancer and Bone Marrow Transplantation of Wroclaw Medical University.*

Streszczenie.** W artykule przedstawiono wyniki badań in vitro przeprowadzone na stanowisku badawczym zaprojektowanym i zbudowanym na Politechnice Wrocławskiej w Laboratorium Badania Zagrożeń Elektromagnetycznych i Bioelektromagnetyzmu mające na celu ustalenie wpływu stałego pola magnetycznego na poziom apoptozy komórek białaczki ludzkiej HL-60. W artykule zaprezentowano uzyskane dotychczas wyniki z badań medycznych przeprowadzonych na Uniwersytecie Medycznym we Wrocławiu w Katedrze i Klinice Hematologii, Nowotworów Krwi i Transplantacji Szpiku. **Badanie wpływu stałego pola magnetycznego na apoptozę komórek białaczki ludzkiej HL-60.

Keywords: constant DC magnetic field, in vitro, HL-60 cell, apoptosis.
Słowa kluczowe: Stałe pole magnetyczne, in vitro, komórki białaczki ludzkiej HL-60, apoptoza

Introduction

The Earth's magnetic field

The human body in the environment in which it lives, is permanently exposed to constant (DC) electric and magnetic fields and to electromagnetic waves. Both the electric and magnetic fields are states of space, in which complex phenomena take place (also in a vacuum – hence, as we can see, the vacuum is not always "empty").
Electric and magnetic fields can be seen as one of the basic energy forms known to man. Since its inception the life on Earth has been evolving in a constant magnetic and electric field.

Earth's magnetic field occurs both within and around it, spreading to the many thousands of kilometers from its surface, creating an area called the magnetosphere. This sphere is a kind of "protective coat" from cosmic rays, and is essential for most processes taking place on the planet on which we live including most likely a variety of processes taking place in living organisms.
On the Earth the values of the surrounding magnetic field depend to a large extent on the latitude and the specific conditions related to the terrain on which it occurs. For the latitudes corresponding to the Poland's position, its value is at ca. 40A/m and in contrast to the electric field it is not directly dependent on the weather [1, 2, 3] .

The Earth's magnetic field is characterized by a frequently met occurrence of magnetic anomalies. These are the local deviations of actual values of the earthly intensities of the magnetic field in a particular location on the Earth from its theoretical value calculated based on the position of the magnetic poles of the planet.

One of the better known magnetic anomalies in Poland is located in the massif of mountain Ślęża and adjoining top Radunia. The reason is that the Radunia hill is built, inter alia, in serpentinite with addition of magnetite. The geological composition is responsible for the occurrence of such high magnetic anomalies in this region.

The effect of electromagnetic fields on HL-60 cells

In a study conducted in 2011 at University of Wrocław entitled: "Magnetic field at 50 Hz: its influence on living cells HL-60" the authors have demonstrated the effect of the magnetic field with a frequency of 50 Hz on human leukemia cells [4]. By exposing the human leukemia cell HL-60 in a magnetic field of 50 Hz with induction of 7mT they showed the impact of this field on the spectrines relocation. These proteins that make up the cell membrane cells HL-60 migrated deep into the nucleus what in turn led to the death of test cells. It has been shown that interaction of HL-60 cell with slowly-varying magnetic field can cause apoptic cell death processes.

As the frequency of constant magnetic field application in medicine constantly grows, the question arises: what impact can have strong DC magnetic fields on HL-60 cells? An example would be the use of strong magnetic fields in the diagnosis of medical Magnetic Resonance Imaging (MRI). To improve the accuracy of MRI imaging the increasingly stronger fields, which value can reach 8T are applied. For this reason, it becomes important to determine their potential impact on the human body and the other living matter.

To the most important centers dealing with this type of issues we can include centers located in the United States and Japan [5, 6]. In comparison to the knowledge of the impact of regular electric field which forces act on the surface of molecules, cell membrane, and even around the human body the state of knowledge on feedback effects of magnetic fields having an impact on the course of biological processes in cells remains enigmatic.

Magnetic fields penetrate deeper because the human body is built of matter weakly interacting with this field. The human body is like a "semi-transparent" for magnetic fields because it can penetrate human cells inside and can potentially affect the chemical and biochemical reactions that occur inside them, which is why processes under the influence of this kind of fields in the cell may be subject to change [7].

The impact of electric fields on living matter has different character. Dielectric properties of cell membranes cause, that the fields of this type are retained on the cell surface and does not penetrate to the inside of it [8]. At the same time, it is possible that the cell membrane which in some cases interacts with magnetic and electric fields can act as a kind of antenna [9].

IARC-International Agency for Research of Cancer qualified constant magnetic and electric fields as not

carcinogenic factors [10]. At the same time, it should be noted that despite many studies carried out up to date, the impact of constant fields on human leukemia cells HL-60 is indefinite, still. One has to note that patients suffering from leukemia are frequently exposed to this type of field during MRI tests what makes the assessment of the impact of this type of fields on HL-60 cells becomes an important scientific issue.
This study deals with an attempt to assess the effect of constant magnetic field on the proliferation of cells HL-60 in vitro. Presented material is the first part of the carried out interdisciplinary biomedical research.

Materials and methods used in research

Exposure in the magnetic field

The exposure consisted in placing a sample tube filled with HL-60 cells in a constant magnetic field with 0.5T induction. The research stand was designed and built at the Wroclaw University of Technology in the Laboratory of Electromagnetic Hazard Research and Bioelectromagnetism. The stand is based on a neodymium magnetic element that is the source of a constant magnetic field. The source is made of N48 material with dimensions: length: 60 mm, width: 60 mm, height: 25 mm, direction of magnetization: along the height dimension. The weight of the magnetic element is 674 [g]. The magnetic properties of the source of the magnetic field are as follows: remanence Br 1.38-1.42 [T], normal coercivity HcB min. 835 [kA / m], intrinsic coercivity HcJ min. 875 [kA / m], maximum energy product (BH)max 366-390 [kJ / m3]. The direction of magnetization along the height means that the perpendicular to the height of the magnetic element is the "N" pole and the other opposite perpendicular to the surface of the magnet is the "S" pole. Magnetic induction near the edge of the magnetic surface (maximum) at a distance of 0.7 [mm] is ~ 0.520 [T] [11].

The samples were divided into two groups, the first being subjected to a magnetic field for 72 hours and the other being a control group isolated from the magnetic field.

Measurement of magnetic field induction

Identification and monitoring of the constant magnetic field during the tests were carried out using the meter F.W.Bell 4048. Because of a technical capability of the probes applied with the meter, the measurement of only one component of a magnetic field at a time was possible. The instrument has two exchangeable magnetic field probes that use Hall effect in their operation. The meter provides an accuracy of 0.01 mT on its most sensitive measuring range.

In order to determine the magnetic induction value at a given point, the three measurements for the individual field components: Bx, By and Bz should be done and then the effective value of Bsk induction to be calculated using the dependence (1)

$$(1)\ B_{sk} = \sqrt{B_x{}^2 + B_y{}^2 + B_z}$$

Due to its small size, the measuring probe used with F.W.Bell 4048 meter, allows the measurement of the magnetic field (magnetic induction) at a very close distance to its source.

Cell cultures

Human myeloid leukemia cell line HL- 60 was used in experiments. Cells were kept in the incubator (Nuaire), in standard conditions (37 C, 5% CO2, 100% humidity) in a suspension in cell medium RPMI 1640 (IITD PAN, Wrocław) containing 10% fetal calf serum (Invitrogen , Warsaw) and 100 ug/ml gentamycin culture medium (KRKA- Polska).
24 hours before starting the experiment the cells were fed by adding fresh cell medium with the concentration established at 3-4 x105/ml.

Two hundred thousand of HL-60 cells were suspended in 1 ml of cell medium and exposed within 72 h to magnetic field in 75x12 mm, 5ml polystyrene culture tubes
(Sarsted, Warsaw).

Assessment of apoptosis

Apoptosis of exposed to magnetic field HL-60 line cells was assessed by flow cytometry using annexin V binding test with iodine propidyl (PI) staining.
In the test a phenomenon observed already in the early phase of cell apoptosis is used. It manifests in the loss of asymmetry of cytoplasmic membrane, resulting in exchange of negatively charged reminder of phosphatidylserine between inner and extracellular side of cytoplasm. Annexin –V is in turn, a protein with strong affinity to phosphatidilsterine. While labelled with a fluorochrome (fluorescein isothiocyanate FITC) it binds with membranes of apoptic cells in the presence of calcium ions but does not respond while the cells live.
On the other hand propidium iodide is a coloring agent creating fluorescent adducts with ds DNA - does not stain living cells (with retained integrity of cytoplasmic membrane) but penetrates the nucleus of cells.

In the test a set of Annexin V-FITC Apoptosis Annexion Kit I (Becton Dickinson-Pharmingen, San Diego, USA) was used, following the producer's protocol. In brief, both the HL60 cells after exposure to the field and cells of control cultures were washed once by centrifugation, suspended in binding buffer with higher concentration of calcium ions and incubated for 15 min , with AnnexinV-FITC and propidium iodide (PI) solutions. Apoptosis was measured in PAS (Partec, Germany) flow cytometer separately assessing the percentage of cells binding the annexin V(early apoptosis) and the percentage of cells binding both annexin and PI (advanced apoptosis).
Fifteen thousand cells were acquisited for each measurement.

Discussion of the results of the studies conducted

The aim of the carried out experiments was to examine whether the constant magnetic field on the induction level of 0.5 T causes an increase in the level of apoptosis as measured by flow cytometry based on the annexin V binding test with Iodine propidyl (PI) staining in human leukemia cells HL-60.
Exposure times for each of the experiments were 72 hours. Under normal conditions (control cells) the level of apoptosis was relatively low and only the individual cells showed the apoptic properties (Figure 1, box A2).

Experiments were repeated nine times. Extreme results were rejected from statistical processing. Finally, the results of the seven experiments were taken into account. In each experiment, the level of apoptosis was measured in control cells as well as in cells exposed for 72 hours in a constant magnetic field. Before each intake, the cell suspension was thoroughly mixed.

Table1 contains an illustrative histogram graphically comparing empirical distribution of value ranges for individual levels of apoptosis in HL-60 cells present in the control group (GK), and Table 2 shows the distribution of apoptosis in cell ranges HL-60 occurring in the test group (GB) treated with a constant magnetic field.

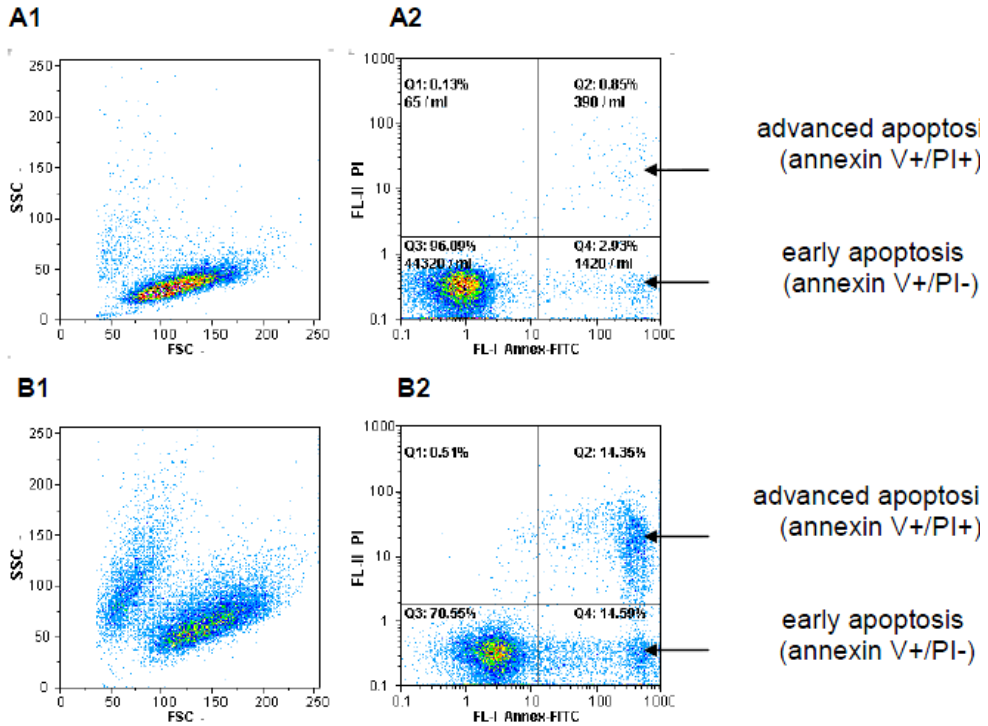


Fig.1. Sample set of cytograms illustrating HL60 cell line apoptosis analysis as measured by cytofluorometry of annexin V binding assay and propidium iodide. Control culture cytograms (upper panel) show low spontaneous apoptosis and culture with induced apoptosis (down). Significant changes in cell morphology (A1 vs. B1) and increase in early apoptotic cells (lower right quadrant) and advanced (right quadrant, upper right quadrant), A2 vs. B2 can be seen.

Table 1. Histogram illustrating the distribution of the results of the apoptosis in the control group (GK). Black boxes illustrate the number of results in the individual value ranges of registered values.

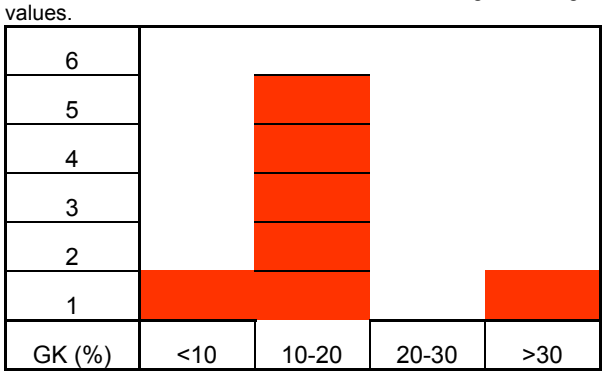
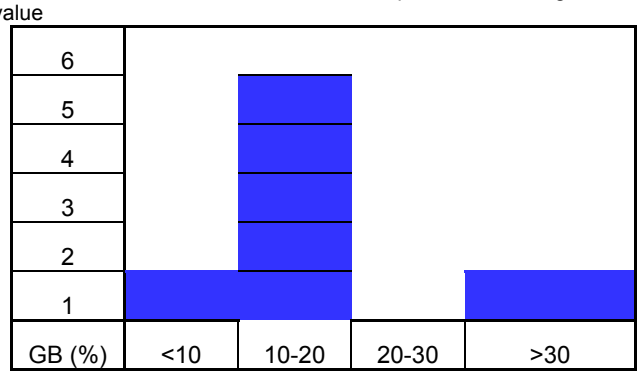


Table 2. Histogram illustrating the distribution of the results of the apoptosis in test group (GB). The black rectangles illustrate the number of results in the individual compartments of registered value



Each column of tables illustrates the incidence of apoptotic values for each percentage ranges: 0-10%, 10-20%, 20-30% and above 30%. The analysis of the statistical results obtained from the research was based on the interval estimation consisting of a numeric interval design (the confidence interval for the arithmetic mean), that with a probability of 95% will contain an unknown, true value of the estimated parameter from the general population.

Table 3 summarizes the results of the statistical analysis of the experimental research analyzed. The following denotations of statistical distribution parameters were used: $\alpha = 0.05$ - significance level, m - mean value, SD - standard deviation, P - confidence interval.

Table 3 Results of the statistical analysis of apoptosis level in the studied HL-60 cell population before and after exposure in a constant magnetic field 0,5T5T

	Apoptosis level in the control group (GK)	Apoptosis level in the treated group(GB)
M – mean value	12,76	13,67
SD –standard deviation	8,07	6,78
P – confidence interval_+/-	5,0	4,2

Figure 2 illustrates the level of apoptosis under the influence of a constant magnetic field of 0.5T. The chart shows average values, the GK column corresponding to the control group, and the GB column corresponding to the impacted magnetic field group. The calculated confidence intervals for $\alpha = 0.05$ are indicated by vertical black line sections.

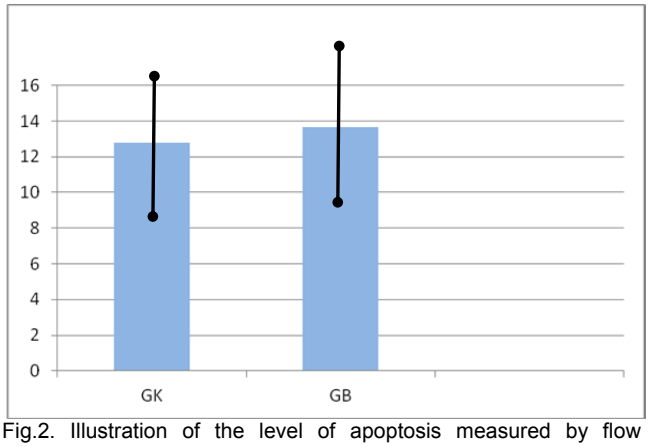


Fig.2. Illustration of the level of apoptosis measured by flow cytometry under constant magnetic field 0,5T. The chart shows average values and confidence intervals for the control group GK and the tested group GB subjected to the magnetic field.

The analysis of the results of observation of the apoptosis level showed no statistically significant differences between the control and tested group. There was no effect of constant magnetic field of the induction 0.5T on the level of apoptosis in the studied population.
At the same time, the analysis of literature from the area of the impact of the constant magnetic field suggests that it may have an effect on intracellular processes [12].
The analysis of the results of the studies conducted and of the literature indicates the need to continue the studies and expand the scope of research on higher sequential values of magnetic fields up to 8T, such as those exposed to imaging with the latest generation of magnetic resonance imaging devices.
These studies may allow for an explanation of the mechanisms of the impact of constant magnetic fields on living organisms, including the human body, which can contribute, inter alia, to assess the response of living organisms as a function of increasing induction level and exposure of constant magnetic field.

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