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Effect of static magnetic field and Busulfan on HL-60 cell apoptosis

***Abstract**. The article presents the results of the in vitro studies which were carried out to determine the effect of constant magnetic field (SMF - static magnetic field) and organic chemical called Busulfan on the level of apoptosis of human leukemia cells HL-60 in the study populations. The mortality increase of cells HL-60 in the case of a constant magnetic field and busulfan with respect to groups of cells treated with cytostatic only has been discovered. The use of a constant magnetic field as a supporting factor in therapy with selected cytostatic has been proposed.*

Streszczenie.** Artykuł prezentuje wyniki badań in vitro których przeprowadzenie miało na celu ustalenie wpływu stałego pola magnetycznego (SMF – ang. Static Magnetic Field) oraz związku chemicznego o nazwie Busulfan na poziom apoptozy komórek białaczki ludzkiej HL-60 w badanych populacjach. Stwierdzono wzrost śmiertelności komórek HL-60 w przypadku zastosowania stałego pola magnetycznego i Busulfanu względem grupy komórek poddanych działaniu samego cytostatyku. Postuluje się zastosowanie stałego pola magnetycznego jako czynnika wspomagającego terapię wybranymi cytostatykami. **(Wpływ stałego pola magnetycznego oraz busulfanu na apoptozę komórek HL-60)

Keywords: static magnetic field - SMF, in vitro, HL-60 cell, apoptosis, Busulfan

Introduction

From the beginning of their existence the living organisms are accompanied by Static Electric Field – SEF and Static Magnetic Field – SMF. Most of living organisms do not have the capabilities of its detection (the exceptions include certain bacteria, insects and birds that take advantage of the constant magnetic field of the Earth for navigation). Magnetic and electric fields are "unnoticeable" for human senses, but can they remain indifferent to its body?

All matter around us in all three states of aggregation manifests both electrical and magnetic properties Each body located in the range of the electric field distorts the distribution lines of this field, which is the result of field interaction with this body. The more the electric field force lines are disrupted, the greater is the interaction between body and the field. All the bodies found in nature have also magnetic properties that are determined by the ratio of magnetic permeability μ. Depending on the value of this factor all the surrounding matter can be divided into three basic groups: ferromagnetic, paramagnetic and diamagnetic materials. Each body placed in a magnetic field distorts the force lines of the field. The size of this distortion depends on the coefficient μ, and is an expression of the impact of this body with the magnetic field. In case of the paramagnetic and diamagnetic materials, for which the value of μ is close to 1, the observed distortions are relatively small. The biggest distortions occur in the case of ferromagnetic bodies, for which $\mu > 1$. Each body entered into the area of the magnetic field obtains the magnetic moment which is called magnetization of the body. Body field interacts with the outer field, so the field does not remain indifferent to the matter in its range [1].

Impact of SMF fields on living organisms

Living organisms are complex systems with solids, gases and liquids. All tissues that make up the living organisms have both electric and magnetic properties. It is difficult to determine how living organisms are "perceived" by the static magnetic field. Most of living organisms consist of water, which has diamagnetic properties however throughout the body one can find many elements of paramagnetic as well as ferromagnetic materials.

The question arises as to whether the constant magnetic field may affect living organisms? All phenomena combine and describe fundamental for electromagnetism Maxwell's equations. Since in living organisms on different levels of biological organization we can see the movement, it follows that even a static magnetic field may be non-indifferent for living organisms in its range. The nature and effectiveness of this type of impact will depend on the value of the induction of this field and its uniformity. In the tissues moving in a constant magnetic field or staying in an alternating magnetic field the E.M.F. and currents will be induced. In addition, under the influence of external electromagnetic field on living organisms we can observe inter alia, [2.3]: magnetization, the impact on diamagnetic molecules as well as on liquid crystals in the body, the effects on the water, impact on depolarization of cells, piezoelectric and magnetostrictive elements. Thus we can observe the impact of constant magnetic field on the living organisms but precise definition of its impact is very complex.

Magnetic fields in medical applications

Magnetic fields are used for decades in the medical therapy (among others, in magneto therapy, magnetic stimulation and transcranial magnetic stimulation -TMS reaffirming that under certain conditions, this physical factor may have influence on the processes in organisms subjected to its impact [4]. At the same time, in recent decades, we see continuous growth of its use in technology and medicine. Therefore a need arises to research the impact of static magnetic fields- SMF on the living organisms at different levels of biological organization, which will identify more accurately the potential impact of that physical factor on the matter which is under its influence. Fields of this type are used in the medical diagnostics, for example in imaging devices presenting the interior of the human body, so called magnetic resonance imaging – MRI devices. Increasingly newer devices of this type apply the higher values of the constant magnetic fields needed to secure better resolution of obtained images. Literature review of experiments results in this area which were carried out so far indicates that, under certain conditions, this type of physical factors can also affect physiological processes occurring in the human body. In living organisms, there are many structures of magnetic moments. Therefore, it can be expected that the static magnetic field (SMF) may have an impact on the metabolic activity of organisms/cells. So that, this type of field may have a potential impact on the course of catabolic and anabolic response what may affect the speed of metabolism [5]. In the year 2011 the effect of the magnetic field with a frequency of 50 Hz on human leukemia cells was shown. By exposing the HL-60 cells in alternating magnetic field with the induction of 7 mT the impact of this field on the spectrine relocation has been demonstrated. The proteins that make up the cell membrane in HL-60 cells migrated deep into the nucleus which in turn led to the death of test cells. It has been proven that impact of slowly – varying magnetic field on HL-60 cells can cause apoptic processes in them [6]. On the other hand in the year 2016 the impact of the SMF on the apoptosis level in cells HL-60 has been tested. Test cells HL-60 were subject to the influence of the SMF field with a value of 0,5T for a period of 72 hours. Results from the studies conducted did not show increased mortality in the study populations relative to the control group, which was not exposed on the operation of this SMF. There was no effect of static magnetic field with induction 0.5 T on the change of the level of apoptosis in the population [7]. This suggests that people who are sick of leukemia can be subjected to MRI imaging of such field values of the SMF without the potential impact on the course of the cancer process. At the same time, the question arises as to whether such field values influence the course of cancer processes in humans treated with chemotherapy agents. Patients suffering from leukemia treated with cytostatic are often exposed to this type of field during MRI tests. For this reason, an important scientific issue becomes the assessment of the impact of this type of fields on cell HL-60 that are simultaneously exposed to chemical factors and the physical ones like SMF. The studies in this field, carried out so far, suggest the potential to increase the effectiveness of action of some chemical compounds in the fight against cancer by the coexistence of SMF [8,9]

In the year 2016, the researchers conducted a study using permanent magnet to produce static magnetic field-SMF what unequivocally denied the possible effect of temperature on the conducted experiments. However, in this case the authors have mentioned only that the experiments were carried out by magnetic induction of 1 T but have not provided sufficiently accurate source parameters of applied magnetic field to allow for replay the same experiment in another research laboratory [10]. Also, the results of other studies of this range might be burdened with faults caused e.g. by temperature influence that could have a significant impact on the course of the experiment. If a static magnetic field is generated in the set of coils by which the current flows, the local impact of temperature on the outcome of the experiment can't be excluded [8].The results of our tests presented hereafter are devoid of such problems.

Materials and methods used in the studies

Exposure in static magnetic field

The exposure consisted in placing a test tube filled with HL-60 cells in a static magnetic field of the induction 0.5 T. Testing stand to the exposure cells HL-60 applied in the experiment is depicted in Figure 1. It consists of base 1 on which a neodymium magnet 2 was placed, on which the tube 4 was placed where there were cells HL-60 number 5. Tube is placed in the rack 3. The source of the magnetic field is a neodymium magnet which is made of N48 with dimensions: length: 60 mm, width: 60 mm, height: 25 mm, the magnetization direction: along the dimension of 25 mm. Weight of the magnetic element is 674 [g].

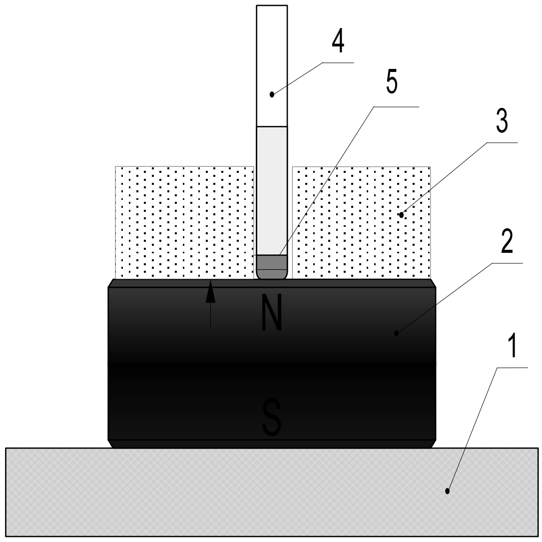

Fig.1. Testing stand for exposure of HL-60 cells applied in experiment. On the base marked 1 the neodymium magnet 2 was placed, on which the tube 4, fixed in the rack 3, was located. Tube was filled with tested HL-60 cells 5.

Magnetic properties of the source of the magnetic field are as follows: the remanence Br 1.38-1.42 [T], normal coercivity HcB min 835 [kA/m], intrinsic coercivity HcJ min 875 [kA/m], magnetic energy density (BH) max 366-390 [kJ/m3]. The direction of magnetization along the height means that the surface of magnetic element that is perpendicular to the height forms the pole "N" and its counterpart on the opposite end of the magnet forms the "S" pole. Magnetic field induction close to the edge of the surface of the magnetic pole (maximum) with a distance of 0.7 [mm] is ~ 0.520 [T] [11].

Measurement of induction of the magnetic field

To identify and monitor the static magnetic field during the research the meter F. W. Bell 4048 was used.

Due to applied type of the probe the meter can measure only one magnetic field induction component at a time. The instrument has two interchangeable magnetic field probes which employ the Hall Effect in their operation. The meter ensures the measurement accuracy 0,01mT on its most sensitive measuring range. In order to determine the value of the magnetic induction at a given point, the three measurements for the individual field component Bx, By and Bz should be done and then the effective induction value Bsk to be calculated. Due to its small size, the measuring probe used with F.W.Bell 4048 meter, allows the measurement of the magnetic field (magnetic induction) in the very near distance from its source.

Cell cultures

Human cells of myeloid blast national cancer line HL-60 were used in experiments. The cells were kept in suspension in the incubator (Nuaire), in standard conditions ($37\ ^0$ C, 5% CO2, 100% humidity) in the culture medium RPMI 1640 (IITD PAN, Wrocław) which contained 10% serum fetal calf (Invitrogen, Warsaw, Poland) and 100μg/ml gentamicin (KRKA-Poland, Warsaw, Poland).

24 hours before starting experiment the cells were fed by adding fresh background while ensuring the cells concentration 3-4 x10 5/ml. Each tested group consisted of two hundred thousand HL-60 cells in a volume of 1 ml of medium kept in 5 ml polystyrene tubes for breeding (75x12 mm) provided by Sarstedt (Warsaw, Poland). In groups which were influenced by cytostatic its concentration was 12μg/ml.

Evaluation of apoptosis

HL-60 cell apoptosis treated with magnetic field was measured by flow cytometry based on Annexin V binding test and propidium iodide (PI) staining.

Annexin V is a protein with a strong

affinity to phosphatidylserine. Marked with a fluorochrome (FITC fluorescein isothiocyanate,) connects in the presence of calcium ions with the membranes of apoptotic cells, but does not respond while the cells live. In turn, propidium iodide is a food dye to create fluorescent adducts with double stranded DNA - does not stain living cells (cytoplasmic membrane integrity of flesh), but penetrates to the nuclei of the cells. The test uses a phenomenon observed already in the early stages of apoptosis which consists in the loss of asymmetry of cytoplasmic membrane what results in transferring the negatively charged residues of phosphatidylserine from the interior to the outer layers of the cytoplasm. The test was carried out with application the Annexin V-FITC Apoptosis Detection Kit (Becton Dickinson-Pharmingen, San Diego, USA) according to the protocol of manufacturer. In brief, the cells after exposure to the magnetic field and the cells from control culture have been centrifuged, suspended in a binding buffer with higher concentration of calcium ions and incubated for 15 min with Annexin V-FITC and propidium iodide solution.

Apoptosis was measured in the flow cytometer (PAS, Partec, Germany). Independent assessments of percentage of cells binding only Annexin V (early apoptic) and Annexin V and PI (late apoptic) were done. Each time 15000 cells were analyzed.

Discussion of the results of the studies conducted

The potential use of a static magnetic field in cancer chemotherapy is a fascinating area giving new treatment options for this type of disease. Analysis of the literature suggests that the SMF field can have an impact on the effectiveness of chemotherapy agents [8,10]. Therefore, the aim of the study was to determine the impact of static magnetic field with induction level 0,5T on effectiveness of cytostatic Busulfan in generating the apoptosis of cancer cells HL-60.

In each of carried out experiments the HL-60 cells were divided into three groups. The first group consisted of cells

that were not subjected to the SMF or to Busulfan. The second group was the cells that were exposed to the chemical compound called Busulfan. The third group consisted of the cells exposed to two factors: Busulfan in concentration 12μg/ml and the SMF field. Each of the experiments was run for a period of 72 hours.

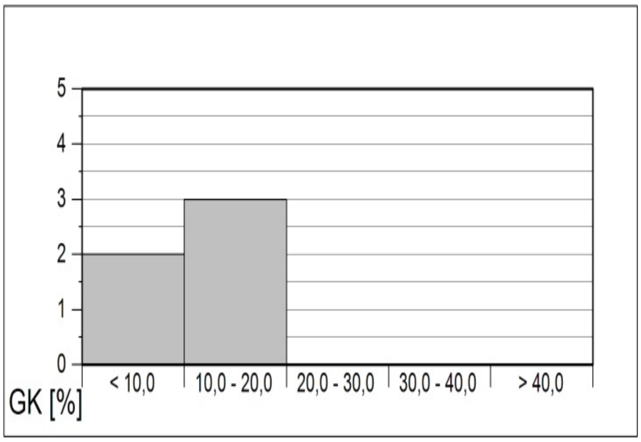

Fig 2.Histogram illustrating the apoptosis distribution in control group GK. Black rectangles show the number of results obtained in given value range

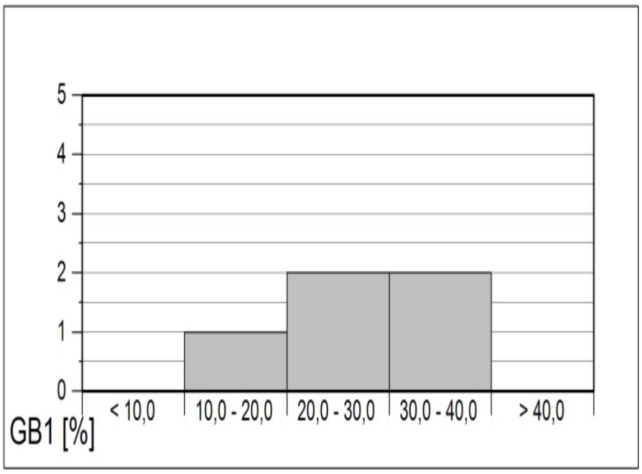

Fig 3. Histogram illustrating the apoptosis distribution in control group GB1 exposed to Busulfan. Black rectangles show the number of results obtained in given value range

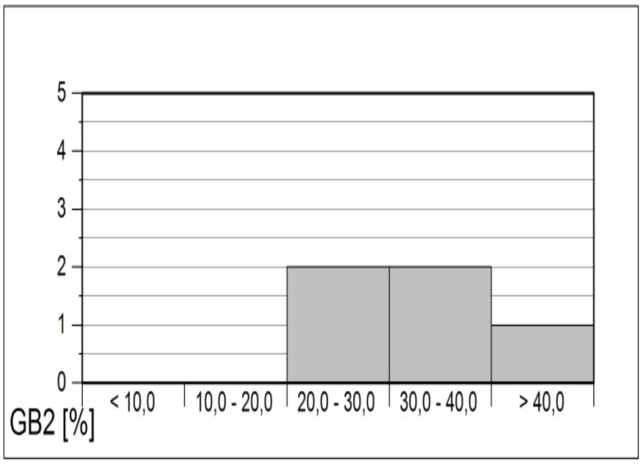

Fig 4. Histogram illustrating the apoptosis distribution in control group GB1 exposed at the same time to Busulfan and SMF. Black rectangles show the number of results obtained in given value range

Account has been taken of the results of the five series carried out experiments. Figure 2 contains the histogram that illustrates in a graphical way empirical distribution of apoptosis incidence in given percentage ranges in HL-60 cells occurring in the control group (GK) while Figure 3 shows the distribution of apoptosis incidence in given ranges in HL-60 cells found in the test group (GB1) treated only with cytostatic. Figure 4 summarizes the empirical distribution of apoptosis incidence in given ranges in HL-60 cells found in test group (GB2) which was simultaneously under impact of cytostatic and SMF. Each column of tables illustrates the incidence of apoptotic values specific for each percentage range: 0-10%, 10-20%, 20-30%, 30-40% and above 40%. The statistical processing of results obtained from the research was based on the interval estimation, which consists in a numeric range 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, what is illustrated in Figure 5. This diagram illustrates the level of apoptosis under the influence of a static magnetic field - SMF with a value of 0, 5T. The graph shows the average values, column GK corresponds to the control group, and columns GB1 and GB2 correspond to the relevant tested groups. The calculated confidence intervals for $\alpha = 0.05$ are marked by vertical black sections.

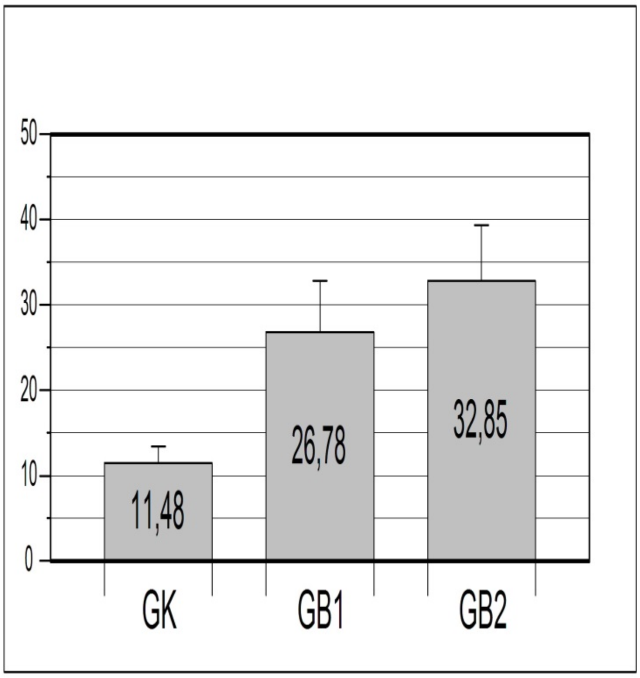

Fig. 5 Illustration of apoptosis level measured with flow cytometry method under the influence of the SMF with a value of 0,5T. The graph shows the mean values and confidence intervals for the control group GK and test groups: GB 1 treated with Busulfan and GB2 treated with Busulfan and SMF.

The analysis of the observation results showed an increase in the average level of apoptosis in the group treated with SMF and Busulfan (GB2 group) of more than six percentage points which gives the increase in mortality of cells by 22,7% comparing to the (GB1 group) not exposed to SMF. The results obtained are consistent with reported in literature influence of SMF fields on living organisms and confirms that such field can have impact on intracellular processes [12].

Conclusions

1. Analysis of the results of the research indicates that there is the possibility to enhance the effectiveness of the treatment with Busulfan by application the SMF fields
2. Analysis of the results of the research implicates the need to continue and broaden the scope of the research to next higher values of SMF field and on another chemicals used in chemotherapy
3. The studies may allow for an explanation of the mechanisms of the impact of SMF 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 value of an SMF and the length of exposure time on the efficacy of cytostatic what can lead to new more effective cancer treatment procedures with the use of these chemicals.

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