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Effects of static magnetic field on human leukemic cell line HL-60

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Abstract

A number of structures with magnetic moments exists in living organisms that may be oriented by magnetic field. While most experimental efforts belong to the area of effects induced by weak and extremely low-frequency electromagnetic fields, we attempt to give an attention to the biological effects of strong static magnetic fields. The influence of static magnetic field (SMF) on metabolic activity of cells was examined. The metabolic activity retardation is observed in human leukemic cell line HL-60 exposed to 1-T SMF for 72 h. The retardation effect was observed as well as in the presence of the mixture of the antineoplastic drugs 5 fluorouracil, cisplatin, doxorubicin and vincristine.

Keywords: HL-60; Magnetic fields; Chemotherapy; Cytotoxicity

1. Introduction

From one point of view, living organisms are electromagnetic systems that use electromagnetic fields from protein folding and macromolecular interactions trough membrane functions to the propagation of information in nervous system. Interest in the interaction of electromagnetic field with living organisms has been triggered primarily from epidemiology studies, which have reported weak associations between magnetic field exposure and a variety of cancers. Therefore, research efforts were mainly focused on weak and extremely low-frequency fields. However, the present experiment is concerned with possible influence of relatively "strong" static magnetic field (SMF) on metabolic activity of cells. Concerning strong SMF, growing demand for the study of biological effects is generated by spreading magnetic resonance imaging system used for medical diagnosis and the probable introduction of new technologies such as magnetically levitated trains.

Electromagnetic fields have been used for decades in medical therapy, confirming that under certain conditions non-ionizing electromagnetic energy can influence physiological processes in organism. Coupling magnetic field exposure with possible chemotherapy of cancers is a new fascinating area that has been evolving in recent years. Current evidences suggest that cell processes can be influenced by the combination of magnetic fields and drugs. Exposure of mice by low-frequency pulsing electromagnetic field increased the bone marrow toxicity of cyclophosphamide [1]. In an in vitro cell growth assay, carboplatin potency against human cancer cell lines A-431 and HT-29 increased after 1-h pulsed magnetic field (PMF) exposure with an average field strength of 0.525 mT. The potentiating effect was not observed with cisplatin. Daunomycin was potentiated only against HT-29 [2]. Cisplatin, carboplatin and doxorubicin had an increased tumoricidal effect when the whole organism of a mouse xenograft cancer model was exposed with the same field as it was mentioned above. The mean tumor volumes in mice treated with combination of drug and pulse magnetic field was 52%, 34% and 35% of that found in the respective cisplatin, carboplatin and doxorubicin drug alone groups [3].

The modulating effects of magnetic field on the efficacy of cancer chemotherapy may find a particular role even in as serious problem as the resistance of tumor cells to many anticancer drugs. Exposure of KB-Ch-8-5-11, a multidrug resistant human carcinoma subline, which over-expresses P-glycoprotein and is resistant to colchicine, daunorubicin, doxorubicin, vinblastine and actinomycin D, to PMF-enhanced potency of daunorubicin only when PMF expo-

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sure occurred in the presence of drug. It has been suggested that this phenomenon is related to the inhibition of the efflux pump, P-glycoprotein [4].

The actions of combination of a SMF and antineoplastic drugs on cellular processes are virtually unknown up to this time. The present study was undertaken to examine an influence of SMF on metabolic activity of widely employed HL-60 human leukemic cells as a model system. The purpose of this investigation was to evaluate whether static magnetic field could modulate the potency of antineoplastic drugs in similar way as pulsed magnetic field.

2. Materials and methods

2.1. Cell culture

HL-60 promyelocytic cell line was kindly provided by Dr. M. Hajduch (Palacky University). This cell line was maintained in RPMI 1640 (Gibco, Great Britain) containing 10% fetal calf serum, 25 mg/100 ml glutamine and penicillin/streptomycine (100 I.U. ml^{-1} and 100 μg ml^{-1}, respectively).

2.2. Metabolic assays

For an assessment of the cytotoxic effect of tested agents, the thiazolyl blue (MTT) method was used in all experiments [5]. Briefly, cell suspensions containing 3×10^4 viable cells per vial were cultivated in 96 well tissue culture plates (Falcon Becton-Dickinson, USA) with or without tested drugs in final volume of 100 μl for 72 h. The cultivation was performed at 37 °C in a humidified 5% CO_2 atmosphere. After 72 h of cultivation, MTT (Sigma, USA) was added to each sample and the cultivation was continued for additional 4 h. During this period, the living cells were produced from the yellow soluble MTT blue insoluble formazan forming microscopic crystals in the cell culture. The reaction was stopped by an addition of 10% lauryl sulfate (Sigma) into each well, and the content of the wells was spontaneously dissoluted within the following 12 h, thus allowing the measurement of optical density. The optical density of each well was measured spectrophotometrically at 540 nm in an ELISA reader MRX Dynatech (Great Britain). The obtained values were calculated and expressed as percentage of metabolic activity in comparison with controls taken as 100% metabolic activity.

2.3. Magnetic exposure

Cells are exposed to SMF in an apparatus made in the authors' laboratory (Fig. 1). The static magnetic field was generated by a pair of coils that are coaxial with iron core in an approximate Helmholtz configuration made of copper wire wound around ring form. The coils were driven by custom-made DC power supply. One disadvantage of large

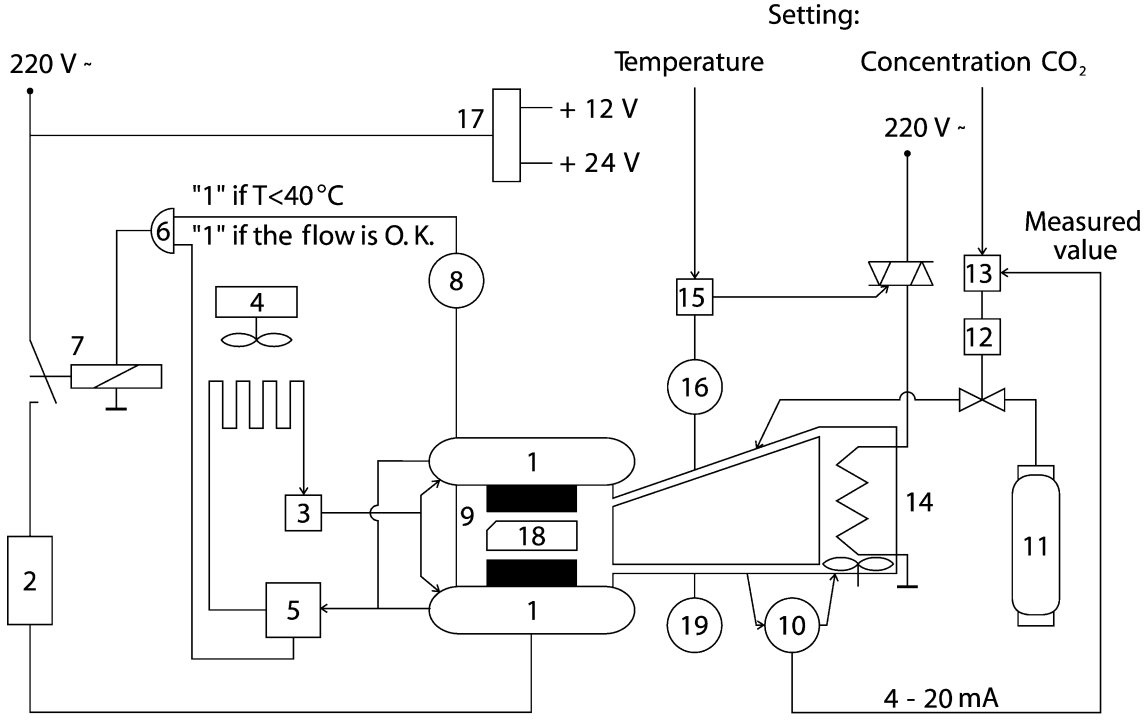

Fig. 1. Principle scheme of apparatus (regulation of temperature and concentration of CO_2). 1: electromagnet, 2: power supply, 3: pump, 4: chiller, 5: flow transducer, 6: AND element, 7: relay, 8: temperature transducer, 9: area of the cuvette, 10: CO_2 meter, 11: CO_2 supply, 12: solenoid valve, 13: CO_2 regulator, 14: heating, 15: temperature regulator, 16: temperature transducer, 17: power supply, 18: cuvette, 19: hygrometer.

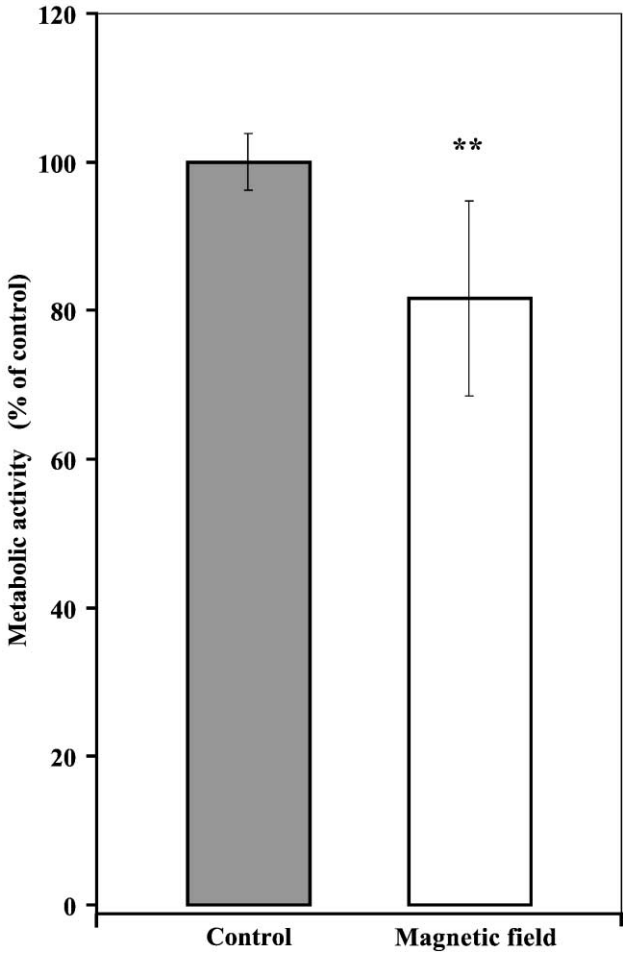

Fig. 2. Effect of SMF on metabolic activity of HL-60 cells. Bars represent the mean ± SD of eight independent experiments. The error margins represent standard deviation (SD). Statistical significance is assessed using Student's *t*-test; $**p<0.01$. The error margin over control bar represents interincubator variability ± 3.7%.

electromagnets is their current consumption that requires cooling systems to reduce heat generated by the magnet coils. Special water-cooled aluminium discs are placed inside coils to isolate a sample from temperature variation during experiments involving long-term exposure. The electromagnets incorporate double overheating control—the detector of flow of cooling water and over-temperature sensors placed on wires of coils both with feedback protective circuits that turn off power supply at a failure of the cooling system.

For the study of samples, strong homogenous field up to 1 T was achieved between ring pole caps as the gap was relatively small (2 cm) compared with the face diameter of the poles (10 cm). Magnetic flux density generated by coils expressed in tesla and homogeneity of field was determined and mapped for nine different points between the magnet ring pole caps by DTM-151 digital teslameter.

Each well with cells was placed within homogeneous part of the magnetic field. The space between coils was enclosed and rebuilt as an incubator, where temperature, humidity and CO_2 were maintained at constant values throughout the exposure period automatically.

Temperature was continuously monitored by thermocouple probe sensitive to 0.1 °C, humidity by hygrometer (± 1%) and CO_2 concentration by CO_2 detector ITR 498 ADOS (Germany). Signals from thermocouple and CO_2 detector, after processing by printed circuit board, switch on/off heat and solenoid valve for automatic supply of CO_2. Sensors of laboratory thermometers were placed in three different points around the jacket for sample inside of the incubator for continuous visual verification. Control cultures were maintained in different conventional incubator SANYO MCO-17AI.

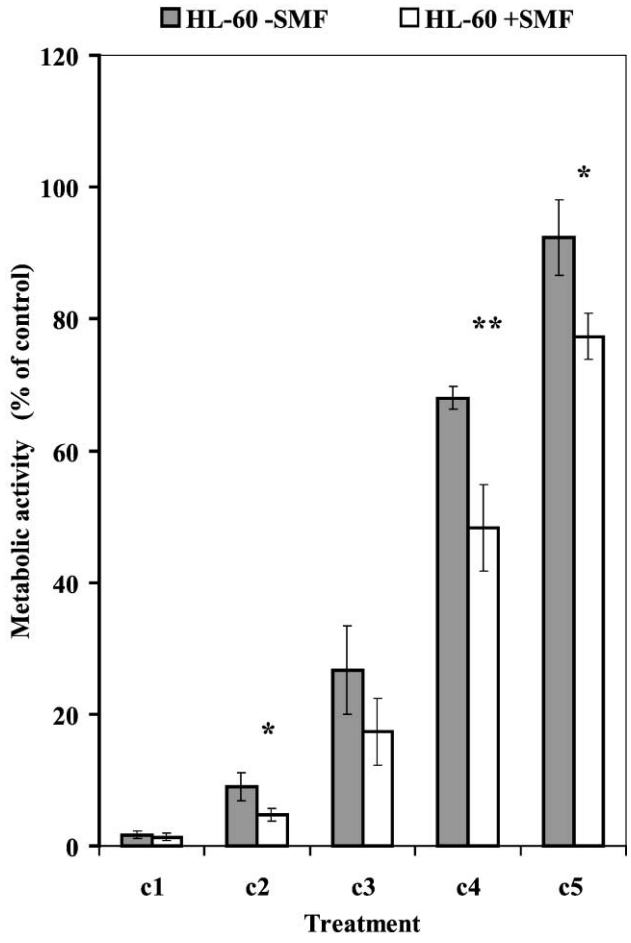

Fig. 3. Effect of SMF on the potency of the mixture of antineoplastic drugs 5 fluorouracil (5FU), cisplatin (C), doxorubicin (D) and vincristine (V). Bars represent the mean ± SD of three independent experiments. The error margins represent standard deviation (SD). Statistical significance is assessed using Student's *t*-test $*p<0.05$, $**p<0.01$. Concentration of drugs in the mixture: c_1 (C = 170 mM, 5F = 154 mM, D = 3.45 mM, V = 54 mM), c_2 (C = 42.5 mM, 5F = 38.5 mM, D = 863 nM, V = 13.5 mM), c_3 (C = 10.6 mM, 5F = 9.63 mM, D = 216 nM, V = 3.38 mM), c_4 (C = 664 mM, 5F = 601 nM, D = 13.5 nM, V = 210 nM), c_5 (C = 166 nM, 5F = 150 nM, D = 3.38 nM, V = 53 nM).

3. Results and discussion

We evaluated the effect of SMF exposure at field strengths of 1 T on metabolic activity of human leukemic cell line HL-60.

Cells were taken from a single "parental" flask and placed into control and exposed plates. Control cultures were maintained during exposure in different incubator. Conditions for growth (temperature, 5% CO_2, humidity) in control and SMF incubators were identical. To rule out a possibility of interincubator artifacts, a series of seven experiments was performed. For each experiment, plates with cells were placed in both incubators and after 72 h, the metabolic activity by MTT test was determined. Intraincubator variability equal interincubator variability of metabolic activity (SD = 3.97%, error bars over control in Fig. 2).

We firstly investigated the influence of SMF exposure at field strength of 1 T on metabolic activity of HL-60 cells. The results of eight replicate experiments are shown in Fig. 2. Application of 1-T SMF for 72 h on HL-60 cells retarded metabolic activity to 81% ($p < 0.01$).

The metabolic activity assay was used to investigate the modulating effect of the combination of static magnetic field and the mixture of antineoplastic drugs: 5 fluorouracil, cisplatin, doxorubicin and vincristine. In Fig. 3, it can be seen that SMF enhanced the cytotoxic effect of antineoplastic drugs. The HL-60 cells were treated with varying concentrations of the mixture of drugs and exposed to 1-T SMF. Metabolic activity was assessed after 72 h by MTT test. The mixture of antineoplastic drugs, depending on concentration applied, significantly decreased metabolic activity of cells in the absence of magnetic field. If applied with static magnetic field, significant differences were found. SMF enhanced cytotoxic effect of antineoplastic drugs in all concentration used. The suppression of metabolic activity was significant in concentrations c_2 ($p = 0.0116$), c_4 ($p = 0.009$), c_5 ($p = 0.019$). As the measurement of metabolic activity using the MTT assay is directly proportional to the cell number [5], we suppose that the decrease in metabolic activity reflects the concomitant decrease in cell number.

Our results show that SMF had a pronounced effect on metabolic activity of human leukemic cell line HL-60. A number of studies have dealt with nonthermal interactions of electromagnetic fields with cells [6,7]. In vitro, exposure of murine immune cells to 0.025–0.15-T SMF decreased macrophage phagocytosis and enhanced apoptotic death of thymocytes [8]. If indeed SMF can influence the basic cellular processes, then it is evident that fast growing malignant tumor cells should be affected more than the normal cells, which could be of particular significance to cancer therapists.

It is unknown why cell growth would be suppressed by the presence of SMF. Magnetic fields of more than 1 mT can have measurable effects on the lifetime of radicals [9]. Chemical reactions are decelerated if the final state has smaller susceptibility than the initial state [10]. According to theoretical studies, a field of 0.5 T could decrease a reaction by 0.2% and if a given biochemical reaction chain consist of 15 reactions, then overall reduction of the final products would be about 20%, which is in rough agreement with our experimental data recorded when 1-T SMF is used [14]. Affected rates of biochemical processes could be linked to enhancement of lipid peroxidation [11], modulation of transcription, increased activity of ornithine decarboxylase and free calcium concentrations in cells [12]. Transformations on molecular level could result in apoptosis [13] or enhanced cytotoxicity of antineoplastic drugs.

Regardless of the unknown mechanism, the data presented in the current report can help pave the way for testing possibilities of therapeutic uses of magnetic field. Moreover, understanding the biological effects of strong magnetic fields and their operating mechanisms would enhance chances for elucidation of possible causal relationship of extremely low-frequency electromagnetic field and cancer, which is naturally a cardinal concern of research in the field at present.

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