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Effects of sinusoidal 50 Hz magnetic field on viability, cell cycle and apoptosis of HL-60 cells

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Abstract. This study was undertaken to investigate whether low frequency magnetic fields can affect the characteristic of cancer cells. To achieve this, HL-60 cells were exposed to 20 mT, 50 Hz sinusoidal magnetic field and the cell viability, cell cycle phase distribution, DNA ladder and apoptotic morphology between control and exposed cells were examined. The results demonstrated that magnetic field had pronounced inhibition effects on cell growth, and small but significant changes in cell cycle phase can be observed. Internucleosomal DNA fragmentation and morphological changes after exposure indicate that the magnetic field induces apoptosis in HL-60 cells. The results also indicate that the growth-inhibitory property of MF on HL-60 cell growth was mainly due to the induction of apoptosis.

PACS. 87.50.-a Effects of radiation and external fields on biomolecules, cells and higher organisms

1 Introduction

In the last few decades, there has been an increasing interest in the biological effects of magnetic field [1,2]. Experimental data suggests that electromagnetic field influences cell functions, including proliferation, apoptosis, morphology and DNA damage [3,4]. Theoretical concepts, such as molecular gyroscopes [5], frequency and amplitude windows [6], ion cyclotron resonance (ICR) [7], etc., have been suggested. New approaches to cancer therapy are needed. It is known, that a malignant tumor cell doesn't possess any qualitatively new metabolic ways, not inherent to the normal tissue cells. With the development of the investigation in the relationship between magnetic field and cancer cells, magnetic field will be of particular significance in cancer therapy.

The inhibition of cell proliferation was observed after magnetic exposure. The ability of static magnetic field and extremely low frequency magnetic field in inducing tumour growth inhibition and cell apoptosis has been reported [8,9]. Exposure of 2 mT, 50 Hz sinusoidal magnetic field for 60 min leads to a reduction in cell number for SV40–3T3 mouse fibroblasts and HL-60 human promyelocytes [10]. Using steep pulsed electric field (SPEF) to kill and inhibit cancer cell and solid tumour is effective. The SPEF contains the extremely wide-frequency spectrum from direct current, low frequency, and high frequency to ultra-high frequency [11]. Low frequency pulsating electromagnetic field combined with drugs or with other physical techniques yields synergistic effect [12,13]. These results are significant for cancer treatment.

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Two human osteosarcoma cell lines exposed under sinusoidal 50 Hz magnetic field show a few significant changes in cell cycle phase distribution [14]. Lange et al. [15] reported that exposure of human amniotic fluid cells (AFC) cells to 1 mT magnetic field had no significant effect on cell cycle. Small variations in cell cycle distribution on SV40-3T3 and HL-60 cells are reported after exposure to 2 mT, 50 Hz magnetic field [16]. However, no effect of magnetic field exposure alone on cell cycle distribution of AFC cells was observed neither at 1 mT nor at lower flux densities [17].

DNA damage checkpoints might become activated in the early stages of human tumorigenesis, leading to cell-cycle blockade or apoptosis and thereby constraining tumor progression [18]. Research has proposed that interaction of electromagnetic fields with electrons in DNA is a plausible basis for activation of DNA [19]. Since such weak fields can accelerate electron transfer reactions, they also stimulate transcription by interacting with electrons in DNA to destabilize the H-bonds holding the two DNA strands together. Exposure to extremely low frequency (ELF) magnetic field induces a low but significant level of DNA fragmentation [20]. Apoptosis has a critical role in determining both cell growth and survival. The process of apoptosis is characterized by internucleosomal cleavage of DNA and gives information about the damage to nuclear DNA.

In order to study the effects of magnetic field on cell proliferation, cell cycle distribution and apoptosis in cancer cells in vitro, human promyelocytic leukemia cells (HL-60) were exposed to 20 mT, 50 Hz sinusoidal magnetic field for 4 days.

2 Materials and methods

2.1 Cell culture

HL-60 cells (human promyelocytic leukemia, ATCC No. CCL-240) were maintained in RPMI 1640 cell culture medium (Gibco, USA) supplemented with 10% fetal calf serum, 2 mM L-glutamine (Gibco), and 0.11 g/l sodium pyruvate. The cell line was purchased from cell bank of Chinese Academy of Science, Shanghai. The cells were grown in 96-well plate or 25 cm^2 T-flasks and maintained at 37 °C in a humidified 5% CO_2 incubator (HERAcell 150, Germany) to be prepared for experiments. Cells were subcultured 24 hours before experiments. Prior to exposure, the cell density was adjusted to 1×10^5 cells/ml.

2.2 Electromagnetic field exposure system

A continuous sinusoidal 50 Hz field generated by a solenoid coils exposure system designed by Department of Electric Engineering (Shanghai Jiao tong Univ. China) was used in the experiments described in this article. Solenoid coils were located within incubator horizontally. The generated magnetic field was vertical. The exposed samples were placed on a Plexiglas slab in the center of the solenoid coils, such that the samples were located symmetrically about the axis of the solenoid coils with the center of the sample halfway between the coils, where the magnetic field is most intense and uniform. The solenoid was connected to a step-down transformer and to a variable transformer with an affixed scale that was plugged in to a 50 Hz, 220 V AC source. The current flowing into coils is observed by a multimeter. When the coils are energized, the 50 Hz sinusoidal magnetic fields can be regulated from 0 to 30 mT.

2.3 Electric field and current density induced by the magnetic field

Low-frequency electric fields do not penetrate cells very effectively because of the low dielectric constant of the cell membrane, but low frequency magnetic fields do penetrate [21]. According to Faraday's Law of Induction, a time varying magnetic field will induce an electric field that is nonuniform in spatial distribution, according to the equation:

$$\oint \vec{E} \cdot \vec{dl} = -\frac{d}{dt} \iint \vec{B} \cdot \vec{dS} \tag{1}$$

$$E(t) = -\frac{r}{2} \frac{dB(t)}{dt} = \pi r f B_0 \sin \omega t \tag{2}$$

where B_0 is the peak value of the magnetic flux density (T), f is frequency (Hz), and r is the radius of a circular contour (m). In the present study, considering radius of 5 cm, the induced electric field E (peak) was 15.7 mV/m at 50 Hz. The induced electric current density (A/m^2) was calculated from the equation $J = \sigma E$. The conductivity (σ) of a typical culture medium is approximately 1.5 S/m [22]. The value computed with the aforementioned formula was 23.55 mA/m^2.

2.4 Exposure protocol

The exposed groups were continuously exposed to a 50 Hz, 20 mT magnetic field. Control groups were incubated concurrently in another incubator with the same conditions but free of exposure. Cells were incubated with or without exposure for 1, 2, 3, or 4 days. Experiments under the same conditions were repeated four times.

2.5 Cell viability assay

Cell viability was assayed by using Cell Counting Kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's protocol. A CCK-8 standard curve was made at first. HL-60 cells were inoculated to 96-well plate at the cell number from 5×10^3 cells to 1×10^6 cells. Cells were incubated for 4 h at 37 °C, 5% CO_2. At the indicated time points, the cell numbers in triplicate wells were measured as the absorbance (450 nm) of reduced WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodim salt). Then a linear relation between cell number and absorbance was calculated.

In following experiment, HL-60 cells were plated in 96-well plates at 1×10^5 cells/ml and continuously exposed to magnetic field for up to 4 days, and viability was monitored every day. The procedures were the same as discussed above.

2.6 Cell cycle analysis

HL-60 cells cultured in T-flask were centrifuged at 2000 rpm for 3 min and supernatants were reserved. Cells were washed with PBS once and permeabilized with 70% alcohol on ice overnight. After cells were washed with PBS again, 500 μl Propidium Iodide solution (RNase A 100 μg/ml, Sigma; PI, 100 μg/ml, Sigma; Triton X-100 0.5%, Shanghai Shize) was added. Cell cycle phase was assessed by FACSCalibur using the MODFIT software (BD Biosciences, USA). For each of the flow cytometry analyses at least 10^4 events were calculated.

2.7 DNA ladder assay

The DNA ladder assay was carried out to detect apoptosis and DNA degradation, and a modified method was used just as Taga et al. described [23]. About 10^6 cells were harvested by trypsinization at day 4. Cells were washed with PBS and lysed at 4 °C for 10 minutes in lysis buffer (10 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 0.5% Triton X-100). After 13000 rpm centrifugation at 4 °C for 30 minutes, the supernatant of cell lysates was treated

with RNase A (400 μg/ml) and proteinase K (400 μg/ml) at 37 °C for 2 hours each. Then NaCl was added to 0.5 M and DNA was recovered by isopropanol precipitation at –20 °C overnight. DNA was dissolved in 10 μl of Tris-EDTA (TE) buffer. A 5 μl DNA sample was electrophoresed on a 2.0% agarose gel and ethidium bromide (EB) staining was used for visualization.

2.8 Hoechst 33258 staining

A modified Hoechst 33258 staining method was used as a necessary assay [24]. At day 4, HL-60 cells cultured in T-flask were collected, washed with PBS, and fixed in 4% paraformaldehyde for 20 minutes at room temperature. After washed with PBS and centrifugation at 2000 rpm for 3 min, the cells were deposited on coverslips and were left to adhere on coverslips for 30 minutes at room temperature. Subsequently, the coverslips were treated with Hoechst 33258 (1 μg/ml) for 20 minutes, rinsed with PBS twice. The cells were viewed with Nikon Eclipse TE2000-S fluorescence microscope.

2.9 Statistical analyses

The results are represented as mean values and standard deviations of these ratios. Additional statistical analyses were made with Student's t test. The statistical differences refer to the relation between exposed cells and control cells at the same time points. Not statistically significant means that $P > 0.05$.

3 Results

3.1 Effect of magnetic field on cell viability

Using the CCK-8 assay, the standard curve was drawn as Figure 1. The number of viable cells was directly proportional to the absorbance. Based on the standard curve, cell density was monitored at fixed interval times (1, 2, 3, 4 days).

Exposure time-dependent inhibition of cell growth by magnetic field was observed in both cell lines examined. Figure 2 report the HL-60 cells growth as a function of time up to 4 days for both exposed and control cells, respectively. As compared to unexposed cells, 4 days' exposure to magnetic field caused cells to grow slowly. HL-60 cells were sensitive to growth inhibition by magnetic field, showing decreased proliferation ability, and the effect was most obvious at day 4. Exposure and control groups reached their maximum cell numbers at day 4. These results demonstrated the potency of 20 mT, 50 Hz magnetic field in inhibiting proliferation of human cancer cells in vitro.

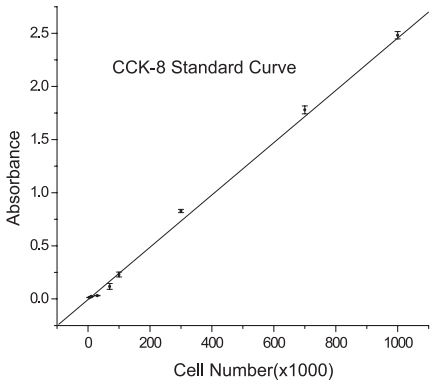

Fig. 1. The standard curve of CCK-8 method. All data points are plotted as mean ±SD ($n = 4$).

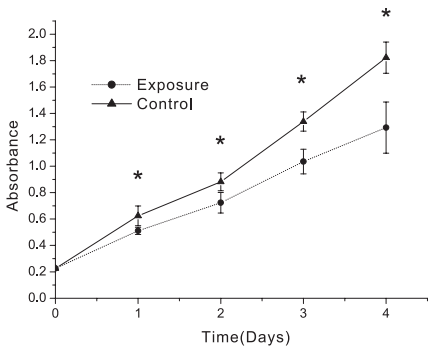

Fig. 2. Effects of 20 mT, 50 Hz magnetic field on the viability of HL-60 cells. Cell viability was determined by CCK-8 method. All data points are plotted as mean ±SD ($n = 4$, * $P < 0.05$).

3.2 Effect of magnetic field on cell cycle

The effects of magnetic field on the cell cycle of HL-60 cells were determined using FACS analysis. Before experiments, the normal distribution pattern of growing HL-60 cells is about 50.51% in G_0/G_1 phase, 37.07% in S phase and 12.43% in G_2/M phase (data not show in the figure). Exposure to 20 mT magnetic field had different effects on the cell cycle of the cells during 4 days, as shown in Figures 3–5. The magnetic field induced a small but significant increase in the length of G_1 phase of cell cycle comparing to control one at 1 day and 3 days. Nevertheless, a tendency to lower G_2 values was observed. The values of exposed cells were always lower than those of control one during 4 days. At the same time, the maximum accumulation in S phase was reached at day 2 after exposure, followed by a decline and remained almost unchanged in the next two days.

No significant induction of apoptosis was observed at 1, 2, 3 days by FACS analysis. But at 4 days, a little apoptosis was observed. The percentage of apoptosis was 0.51, 1.67, 1.04, and 0.83 in the four samples, respective.

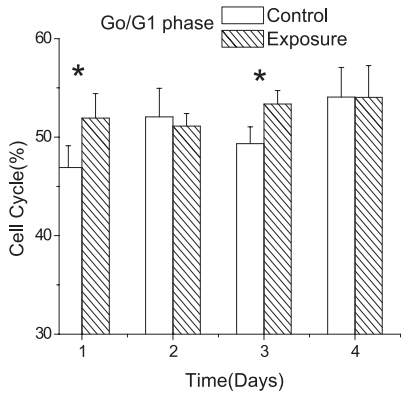

Fig. 3. G_0/G_1 phase of the HL-60 cell cycle. Data are expressed as mean SEM ($n = 4$). * $P < 0.05$.

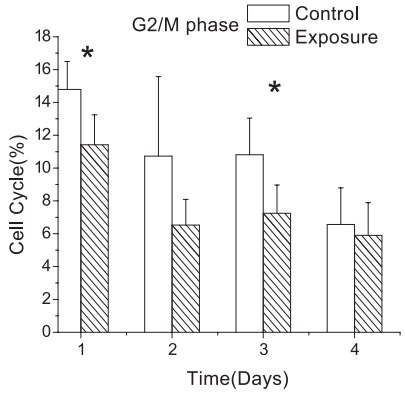

Fig. 4. G_2/M phase of the HL-60 cell cycle. Data are expressed as mean SEM ($n = 4$). * $P < 0.05$.

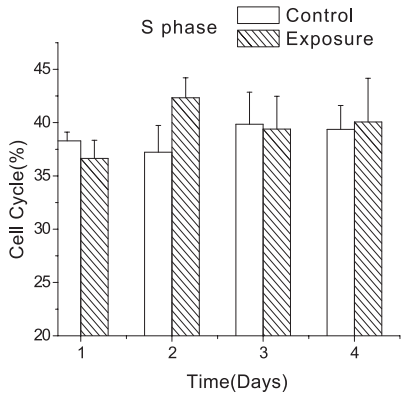

Fig. 5. S phase of the HL-60 cell cycle. Data are expressed as mean SEM ($n = 4$). $P > 0.05$. No statistical difference.

3.3 Detection of internucleosomal DNA fragmentation by DNA ladder assay

One of the features of apoptosis is fragmentation of DNA by endonuclease, which can be detected by DNA ladder assay. This procedure was based on the observation of a distinctive ladder pattern of DNA fragments in agarose gel electrophoresis. It came as a result of DNA cleavage between nucleosomes by an endogenous endonuclease involved in apoptosis [25]. HL-60 cells were exposed to 20 mT, 50 Hz magnetic field exposure and the cells were harvested 4 days later. DNA was analyzed by electrophoresis through an agarose gel. As shown in Figure 6, DNA ladder formation was observed in exposed HL-60 cells, but not in the control one.

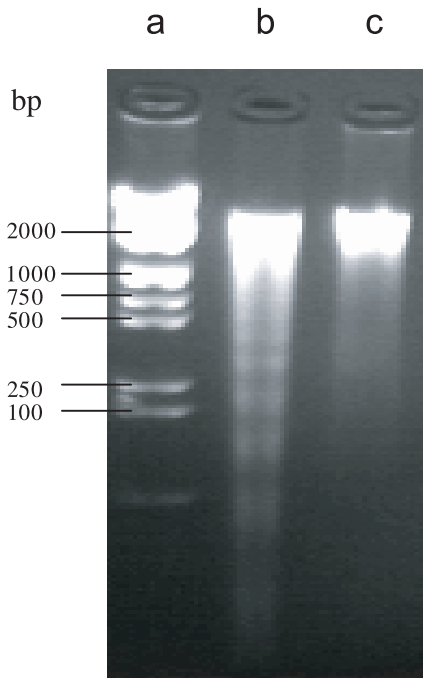

Fig. 6. Fragmentation of HL-60 cell DNA. Lane a: DNA size marker. Lane b: cells after 4 days' 50 Hz, 20 mT magnetic field exposure. Lane c: control cells (free of exposure). One representative experiment out of four is shown.

3.4 Fluorescence microscopy analysis of apoptosis by Hoechst 33258 staining

Hoechst 33258 staining was used to investigate the changes in the nucleus of cells. When exposed to 20 mT, 50 Hz magnetic field for 4 days, the HL-60 cells exhibited condensed and fragmented nuclei, cytoplasmic shrinkage. And in some cells, even the cell membrane was unable to keep intact (Fig. 7). The typical indication of apoptotic cell morphology was observed.

4 Discussion and conclusion

The results showed that magnetic field had pronounced effects on cell growth. Inhibition of cell growth was observed. The same inhibition effect of sinusoidal magnetic field in HL-60 cells has been reported [26]. Small but significant changes in cell cycle phase can be observed during experiments. Besides, characteristic features of apoptosis, such as internucleosomal DNA fragmentation and morphological changes after exposure indicate that the magnetic field induces apoptosis in HL-60 cells.

It is still unclear why the viability of cells will be inhibited by the presence of magnetic field. Liboff et al. report

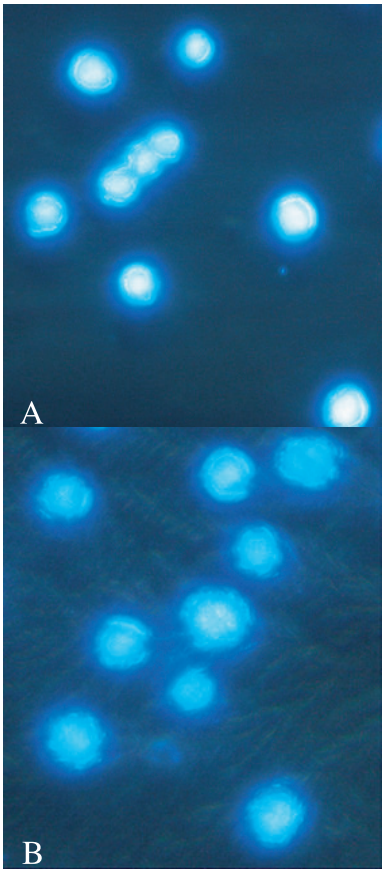

Fig. 7. Morphology of apoptotic HL-60 cells stained with Hoechst 33258 after 4 days' 20 mT, 50 Hz magnetic field exposure. A: control cells (×400); B: exposed cells (×400). Stained cells were examined by fluorescence microscopy.

that sinusoidal magnetic field enhances DNA synthesis of human embryonic foreskin fibroblasts in vitro [27]. He also noted that the proliferation changes were probably correlated with the cell cycle. Our results provide additional evidence to the effect of magnetic field on the cell cycle phase of cancer cells.

Several reports on the induction of DNA damage followed by exposure to different environmental assaults exist. The genomic integrity is analyzed at cellular checkpoints, usually leading to a delay in cell cycle progression after DNA damage [28]. Malignant cells divide and multiply uncontrollably. If indeed magnetic field can influence the basic cellular processes, it is evident that fast growing malignant tumour cells should be affected more than the normal cells, which could be of particular significance to cancer therapists [29]. Efforts have been made to develop a strategy that triggers apoptosis in malignant cancer cells.

Our results suggested that the 20 mT, 50 Hz magnetic field changed the cell proliferation, changed cell cycle phase and induced apoptosis. Furthermore, the results indicate that the growth-inhibitory property of magnetic field on HL-60 cell growth was mainly due to the induction of apoptosis as evidenced by DNA fragmentation and nuclear condensation. The results are not enough. More studies need to be performed to elucidate the mechanisms for the magnetic field inducing apoptosis. Our group will carry out a series of experiments on other tumor cell and normal cell types to further demonstrate the apoptotic potential of the sinusoidal magnetic field exposure in the near future.

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