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Low-frequency magnetic field effect on cytoskeleton and chromatin

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Abstract

The effect of magnetic fields on the living systems is studied in vivo or in vitro in very broad spectrum of organisms, cells and tissues. The mechanism of their acting is not known until now. We studied low-frequency magnetic field effect on cytoskeleton and on the structure of chromatin in human cells. We used cell line of small lung carcinoma (A549) and the effects of magnetic field on cytoskeleton and higher-order chromatin structure were analyzed 96 h of magnetic field exposure. Magnetic field generated by the cylindrical soil was homogenous and the cells were cultivated at 37 °C in humidified atmosphere containing 5% CO_2. Magnetic field induction was B_m=2 mT and the net frequency f=50 Hz. In such affected and control cells the F-actin was estimated using FITC-conjugated Phalloidin and mitochondria were studied using MitoTracker (Molecular Probes). Images of cytoskeleton and genetic loci were acquired using confocal microscopy and analysis was performed by FISH 2.0 software. Slight morphological changes of F-actin filaments and mitochondria were observed in affected cells and nuclear condensation was found. These effects could be related to the process of cell death apoptosis probably induced by magnetic field. The studies aimed at centromeric heterochromatin (9cen) did not show statistically significant changes. Therefore, we suggest that magnetic field has no influence on higher order chromatin structure but certain changes could be observed on the level of cytoskeleton. However, these statements need a thorough verification. Our preliminary experiments will be extended and the effect of magnetic field on another structures of cytoskeleton and cell nuclei will be further studied.

1. Introduction

With the increasing use of electrical appliances in our daily life, most of living organisms including human beings are inevitably subject to the frequent exposures to extremely-low-frequency (ELF, up to 300 Hz) electromagnetic fields (EMF). The mechanism of interaction between ELF-EMF and biological structures, if it exists, is still obscure. Although the results of epidemiological studies on the potentially tumorigenic effects of ELF fields have been largely negative, pooled analyses provide evidence, that prolonged exposures above 0.4 μT is associated with a small risk of leukaemia in children [1–3]. These data have insufficient strength to establish clean conclusion on a relation between EMF and living organisms. Not only macroscopic analyses of cell survival using primary cultures obtained from human and animals show conflicting results but investigations at subcellular level have also failed to explain the alternations observed after treatment with an electromagnetic field source [4]. A lot of papers concerning this topic have been published in the last 25 years. They examined ELF-EMF effects on viability of unicellular organisms [5,6], growth of plants [7], biological effects on gene transcription, changes in synthesis and transcription of DNA [8–10] and effects on the other parts of cells—changes in ion transport [11,12], changes in microtubule organisation [13], production of heat shock proteins [14], etc.

There are varieties of effects that have been described but most of them are only minor and the living organisms seem to tolerate a wide range of electromagnetic stimulation without major effects. That makes this kind of study difficult and it may influence replication experiments with similar conditions of exposure and exposed materials. It is important to perform all experiments under well-defined conditions.

In our previous work we studied EMF effects on viability of different bacterial cells. We found that exposure up to B_m=10 mT, time of exposure up to 48 min, frequency 50 Hz (some experiments had f up to 20 kHz) has negative effect on the growth of bacterial strains *Escherichia coli*, *Staphylococcus aureus* and

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Leclercia adecarboxylata. The metabollic activities of bacterial cultures decreased probably due to lower number of bacterial cells in solution. We stated that the effect of field is probably bactericidal; a part of bacterial cells is dead, the rest develops without problems. The effect is time-dependent and induction-dependent. The acting is also strain-dependent, it seems that stronger effect is observed for rod-like bacteria than for spherical ones [15–17].

In this article we show our results related to the cytoskeleton and higher order chromatin structure of human cell line A549 exposed to magnetic field. Higher order chromosome organization and chromatin modification play an important role in the regulation of nuclear processes. Chromosomes are composed of two different types of chromatin, euchromatin and heterochromatin that occupy discrete domains in the cell nucleus. Transcriptionally active euchromatic compartments are preferentially located in the inner parts of cell nuclei while silent and heterochromatic regions are located near the nuclear periphery [18,19]. Another important subject of higher order chromatin structure studies is active/inactive gene location within chromosome territories. There are several data trying to elucidate this phenomenon. Verschure et al. found nascent RNA in the interchromatin spaces of chromosome territories, which suggested that transcriptionally active regions in chromosome territories are also compartmentalized. Active loci are located predominantly at or near the surface of compact chromatin domains, forming chromosome territories, which enable depositing of a newly synthesized RNA directly into interchromatin spaces [20]. Another data were reported by Kurtz et al.[21], who observed that non-expressed, anonymous fragment was positioned more interiorly within chromosome territory while genes were rather located at the periphery of chromosome territories irrespectively of their activity. In addition, Volpi et al [22] and Mahy et al [23] concluded that gene rich regions involving many active loci can be extended as chromatin loops away from compact chromosome territories.

In our experiments we tested whether magnetic fields could have an influence on nuclear topography of selected chromatin structures. In addition, we tested the effect of magnetic field on selected structures of cytoskeleton such as F-actin filaments and mitochondria. As intracellular organelles, mitochondria show remarkable plasticity, mobility, and morphological heterogeneity [24] and we tested if such dynamic organelles could be affected by magnetic field.

Following our preliminary experiments, we conclude that magnetic field has no influence on higher-order chromatin structure; some changes could be observed on the level of cytoskeleton. These statements will be tested in our further experiments.

2. Materials and methods

2.1. Magnetic field

The magnetic field was generated by a cylindrical coil powered by a transformer. On the coil the applied voltage was $U=21$ V which corresponded to magnetic induction $B_m=2$ mT. The frequency of the applied field was 50 Hz. Cells were exposed to magnetic field for 96 hours in the thermostate temperated to 37 °C, in 5% CO_2 atmosphere. Control samples were kept in the same conditions without magnetic field. The Petri dishes with samples (diameter $r=30$ mm) were kept in the coil on the non-conductive stand. Homogenity of the magnetic field taking in account the sample diameter was about 3%.

2.2. Cell cultivation

Adenocarcinoma cell line A549 obtained from European Collection of Animal Cell Cultures (ECACC, Porton Down, Salisbury, UK) was cultivated in Dulbecco's modified Eagle's Medium (DMEM) (PAN, Germany) supplemented with 10% of fetal calf serum (PAN) and antibiotics (penicillin and streptomycin). Cultivation was performed at 37 °C in humidified atmosphere containing 5% CO_2.

2.3. F-actin detection

A549 cells, growing on slides, were fixed in 4% formaldehyde for 8 min and then washed twice in PBS. Cell permeabilization was performed in 0.1% Triton X-100 in PBS for 8 min and followed washing in PBS for 5 min. Fluorescein-isothiocyanate (FITC)-conjugated Phalloidin (1 g/ml, Sigma) was applied for 45 min at room temperature in the dark. After this incubation, the cells were washed twice for 5 min in PBS and Vectashield (Vector Laboratories, CA) containing TO-PRO-3 (Molecular Probes) was used as a counterstain.

2.4. Mitochondria staining in living cells

MitoTracker (Moleculare Probes, Invitrogene, USA) of the final concentration 200 nM was added to the cell culture growing in fresh medium. After 45 min of incubation, the cells were washed with PBS, fixed with 4% paraformaldehyde for 8 min and nuclei were visualized using TO-PRO-3 (Molecular Probes). Vectashield (Vector Laboratiories, Burlingame, CA) was used as a mounting medium.

2.5. Fluorescence in situ hybridization (FISH)

Cells growing on slides in monolayer were fixed with 4% (w/v) paraformaldehyde (Sigma) in PBS (8 min, RT), washed twice in PBS (2 min, RT). The cells were permeabilised in 0.1 N HCl (v/v), 0.1 M Tris (pH 7.8) and 0.1% (w/v) saponin in PBS, washed in PBS, equilibrated in 20% glycerol (20 min, RT). The following step was freezing the slides in liquid nitrogen and DNA denaturation in 50% (v/v) formamide in 2 × SSC (15 min, 75 °C). Digoxigenin-labelled DNA probe (centromere 8) (Oncor, Gaithersburg, MD) was used for hybridization (18 h, at 37 °C). The post hybridization washing and staining of nuclei was performed according to Bártová et al. [25].

2.6. Image acquisition, analysis and statistical evaluation of the results

The images of immunostained cell nuclei were acquired by a confocal system consisting of argon/krypton laser (Innova 70,

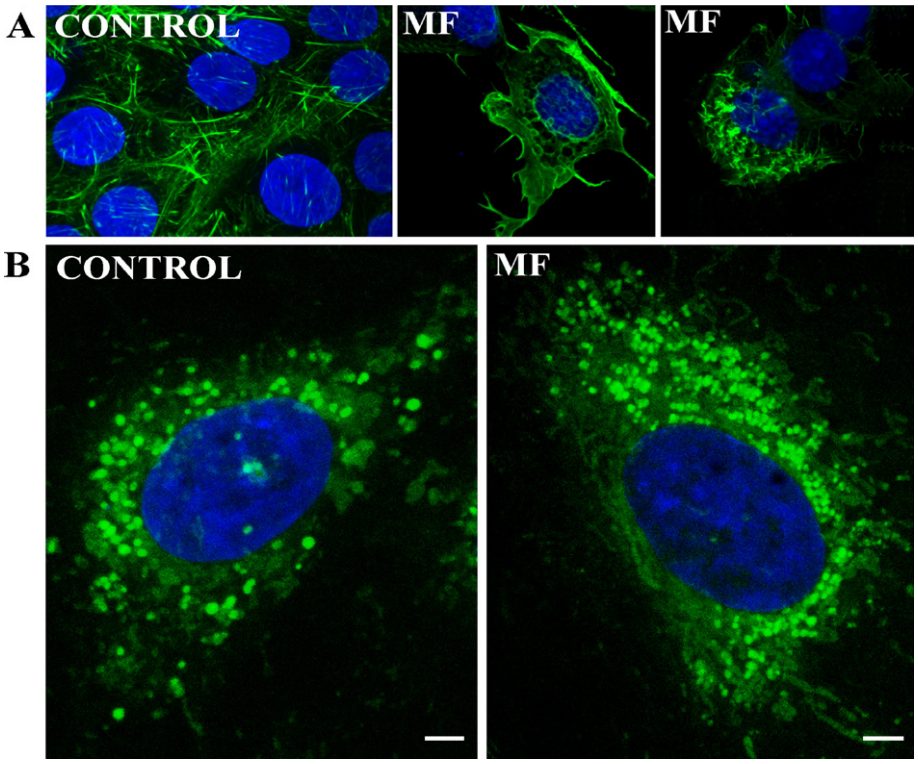

Fig. 1. (A) F-actin filaments in the control (CONTROL) and magnetic field (MF) exposed A549 cells. (B) Organization of mitochondria determined in control (CONTROL) and magnetic field (MF) exposed A459 cells. Bars indicate 1 μm.

Coherent) with acusto-optical tunable filter (AOTF, Brimrose) for the selection of the wave length, confocal head (QLC 100; VisiTech International) connected to a Leica DMRXA epi-fluorescence microscope (LEICA) and Piezzo controlled *z*-movement (Physic Instrumente). The scanning system was driven by FISH 2.0 software [26]. The image system was captured with a fully programmable digital CoolSnap CCD camera (Photometrix, Tuscon, AZ). The magnification of the objective lens was 100x (NA = 1.3). The pixel/micron conversion factor was 15 pixels per 1 μm. For adherent A549 cells, 40 optical sections per 0.1 μm were scanned for each fluorochrome. Maximum image provided complex information about the spatial arrangement of interphase nuclei and cytoskeleton.

Digital images were also analysed using FISH 2.0 software [26] running under MS-Windows environment and containing a number of filters and procedures for image analysis. The simple threshold was applied in order to detect cell nuclei in blue colour. Red fluorescent signals (rhodamin staining) of centromere 8 were detected on a blue background of nucleus by means of a modified watershed algorithm [26,27]. The coordinates of the signals and the distances of centromeres from the nuclear weight centre (CR/R) were computed. All average distances were

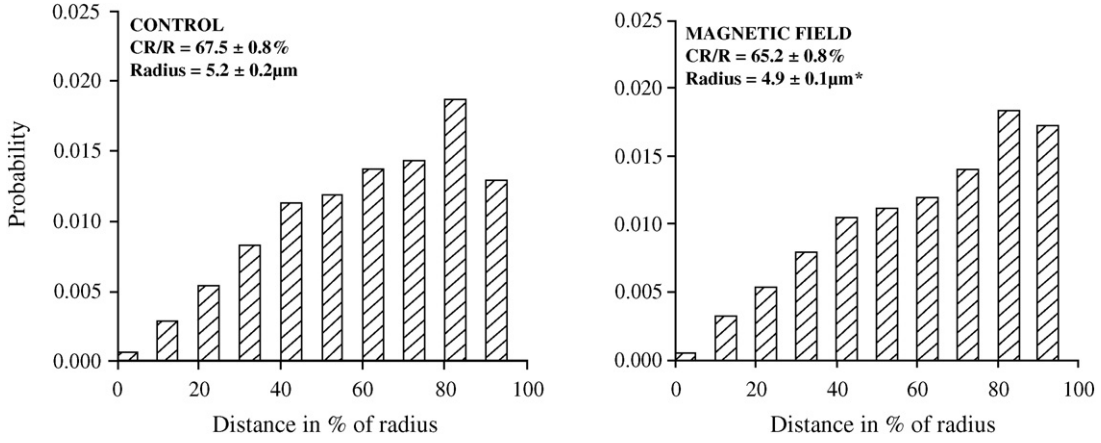

Fig. 2. Nuclear distribution of centromeric heterochromatin of human chromosome 8 in control and magnetic field exposed A549 cells. The distances of centromeres-to-fluorescence gravity centre of nucleus were determined (CR) and normalized to the local nuclear radius (CR/R). * means statistically at $p \leq 0.05$. The Mann-Whitney U-test was used for statistical analyses.

expressed as the percentage of local nuclear radius (R). Analysis of genetic elements was performed automatically and the results of the analysis were confirmed on a screen manually. After the analysis of each image, text files with information about images were generated, imported to SigmaPlot (Jandel Scientific, San Rafael, CA) and further statistically processed. Here, the significance of the differences between distributions of genetic elements was calculated using the Mann-Whitney U-test at $p \leq 0.05$.

3. Results and discussion

3.1. The influence of magnetic field on the structures of cytoskeleton

A549 cells were cultivated for 96 h in the presence of magnetic field. In our experiments we observed reorganization of F-actin filaments (Fig. 1A), which can be the result of the cell death apoptosis that was observed in the cells exposed to magnetic field. Additionally, we observed condensation of interphase nuclei. MitoTracker staining of living cells showed slight reorganization of mitochondria (Fig. 1B). These experiments support the statement that all described effects were closely associated with cell death apoptosis induced. Using DAPI staining we revealed 6.2±1.7% of apoptotic population in magnetic field exposed culture. Moreover, approximately 10% of cells de-attached from cultivation dishes, which is a mark of cell death in the culture growing in the monolayer. This stage was not observed in control cells.

3.2. Higher order chromatin structure of the cells exposed to magnetic field

In order to demonstrate the influence of magnetic field on higher-order chromatin structure, the centromeric regions of human chromosome 8 were studied. It is well known that centromeres are reorganized during many cellular processes such as cell cycle [19], cell differentiation [25,28] as well as in late apoptosis [29]. Following these results we analyzed the arrangement of centromeric heterochromatin in the nuclei of cells exposed to magnetic field. In these experiments, the nuclear positioning of centromeres of chromosome 8 was not changed. We analyzed approximately 300 non apoptotic nuclei and 3D-average distances between centromeres and fluorescence gravity centre of nucleus (CR/R) were 67.5±0.8% in control and 65.2±0.8 % in affected cells (Fig. 2). The differences in the control and treated cells were not statistically significant. On the other hand, statistically significant were the results on the nuclear radius that was 5.2±0.2 μm in control cells and 4.9±0.1 μm in the cells affected by magnetic field (Fig. 2). These values imply induction of chromatin condensation that was caused by magnetic field. It is highly probable, that this chromatin compaction is related to the cell death apoptosis stimulated in magnetic field. It is well known that apoptosis is characterized by chromatin condensation and margination within interphase nuclei [30,31].

It is evident that slight structural changes in the genome could induce its immense functional responses (summary [32,33]) but in our experiments with magnetic field, we did not found changes in the nuclear organization of higher-order chromatin structures such as centromeric heterochromatin. On the other hand, certain effects were observed on the level of cytoskeleton but these changes were rather associated with induction of apoptosis then with the magnetic field alone.

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