Short communication

25 Hz electromagnetic field exposure has no effect on cell cycle distribution and apoptosis in U-937 and HCA-2/1<sup>ch</sup> cells

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Abstract

It is reported that exposure to 50 Hz extremely low-frequency electromagnetic field (ELF-EMF) can produce apoptosis and small variations in cell cycle distribution on different cell lines.

In order to study the effect of ELF-EMF on tumoral cells in vitro, two cell lines (U-937, from a histiocytic lymphoma, and HCA-2/1<sup>ch</sup>, from a human colon adenocarcinoma) were exposed to 25 Hz, 1.5 mT, for 2 h and 45 min. Cell cycle distribution, apoptosis (spontaneous and dexamethasone-induced) and cell growth were evaluated.

Neither significant alteration in cell cycle phases nor induction of apoptosis was observed. Nevertheless, the relative cell number was found to decrease to 55.84 ± 7.35% (p < 0.05, Student’s t-test) for HCA-2/1<sup>ch</sup> cells after exposure to EMF in the presence of dexamethasone.

The presence of dexamethasone during the EMF exposure could probably produce a decrease in the cell growth of this cell line.

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1. Introduction

Electromagnetic fields (EMF) are a non-ionising radiation emitted from household devices, electric power transmission and distribution lines. Public interest in a possible association between exposure to extremely low-frequency electromagnetic fields (ELF-EMF) and cancer risk has increased the number of experimental and epidemiological studies [1]. Many authors have reported a positive correlation between exposure to EMF and the incidence of leukemia, brain tumours and lymphomas [2], but on the other hand, many other studies have failed to discern any correlation between them [3].

The process of apoptosis is characterised by internucleosomal cleavage of DNA and gives information about the damage to nuclear DNA. There are few data concerning the effects of EMF on the mechanisms of cell death or apoptosis. It is reported that exposure to 50 Hz ELF-EMF produces apoptosis in the human myelogenous leukemic cell lines, HL-60 and ML-1 [2]. Simkó et al. [4] observed an increase of induction of apoptosis in SCL II cells after exposure to 50 Hz, 1.0 mT EMF. Ismael et al. [5] noted an increase of dexamethasone (Dex)-induced apoptosis but not spontaneous apoptosis in thymocytes from 0.4 to 1.0 μT 60 Hz field-exposed animals. In addition, other studies have shown that exposure to 2 mT, 50 Hz EMF can produce small variations in cell cycle distribution on SV40-3T3 and HL-60 cells [6].

So far, there have been very few studies on tumoral cells, which investigate the potential effects of EMF with frequencies below 50 Hz. In a previous report, we found significant alterations in cell growth with 25 Hz, 1.5 mT EMF exposure of a human colon adenocarcinoma (HCA) cell line [7].

In the present study, we investigated the possible effects of 25 Hz, 1.5 mT EMF exposure on cell cycle distribution, apoptosis (spontaneous and Dex-induced) and cell growth...
in two human tumoral cells (U-937 and HCA-2/1\textsuperscript{1ch}). This frequency is commonly used in magnetotherapy.

2. Materials and methods

2.1. Cell culture

U-937 cells, a human histiocytic lymphoma, were cultured in suspension in RPMI-1640 medium (with L-glutamine) supplemented with sodium bicarbonate 7.5% (28 ml/l), 10% foetal calf serum and 1% antibiotic–antimycotic solution 100 \times (PSF, Gibco) at 37°C in a humidified atmosphere of 5% CO\textsubscript{2} in air.

HCA-2/1\textsuperscript{1ch} cells, a human colon adenocarcinoma cell line [8], were cultured in Dulbecco’s modified Eagle’s medium nutrient mixture F12-HAM (DME/F12-HAM) (with L-glutamine and hepes) supplemented with sodium bicarbonate 7.5% (28 ml/l), 10% heat inactivated calf serum and 1% PSF (100 \times ) at 37°C in a 5% CO\textsubscript{2}/air atmosphere. These cells grow in monolayer and were subcultured with trypsin (0.05\%) and EDTA (0.02\%) in Dulbecco’s phosphate-buffered saline (PBS) [9].

2.2. Exposure system

The equipment used (Pulsatrón, CEM-84/1; J&J Electromédica) generates rectangular and variable magnetic fields (25 Hz, 1.5 mT peak). Two air core solenoids of 15 \times 10.5 cm were used for the exposure. The cells were cultured in 25 cm\textsuperscript{2} flask and incubated during the exposition at 37°C inside a thermostated box (Thermocult, Boehringer Mannheim). The flasks were placed between the solenoids and isolated to avoid interferences from electronic devices. The magnetic field distribution ran perpendicular to the cell culture surface. Both sample and control sham-exposed cultures were exposed to equal environmental conditions [7].

5 \times 10^3 exponentially growing cells were seeded in each 25 cm\textsuperscript{2} flask. After 24-h incubation at 37°C, 5% CO\textsubscript{2}, the cultures were exposed to 25 Hz EMF, 1.5 mT, for 2 h and 45 min with and without Dex (0.3 µg/ml). Cells were incubated for an additional field-free 24-h period at 37°C, 5% CO\textsubscript{2}, to permit cell growth or death, and then harvested and fixed with 100% methanol.

2.3. Electric field and current density induced by the magnetic field

The magnetic field induces an electric field within the medium that is non-uniform in spatial distribution. The radial dependence of the induced electrical field can be calculated as 
\[ E_{\text{peak}} = \pi r f B \]
where \( B \) is the peak value of the magnetic flux density (T), \( f \) its frequency (Hz), and \( r \) is the radius of the culture (m). In the present study, for a mean culture radius of 2.7 cm, the induced electric field was 3.18 mV/m at 25 Hz. The induced electric current density (A/m\textsuperscript{2}) was calculated from the equation \( J = \sigma E \). The conductivity (\( \sigma \)) of a typical culture medium is approximately 1.5 S/m [10]. The value computed with the aforementioned formula was 4.77 mA/m\textsuperscript{2}.

2.4. Cell cycle distribution and apoptosis

Effects on the cell cycle and apoptosis were measured by means of flow cytometry (FACScan, Becton Dickinson, San Jose, CA). Cell cycle distribution, spontaneous apoptosis and Dex-induced apoptosis were assessed by the analysis of DNA and the detection of DNA fragmentation [5] after lysing cells in a hypotonic solution (0.1% sodium citrate, 0.1% Triton X-100) containing 125 µg/ml propidium iodide (PI).

At least 3 \times 10^4 cells from each sample were analysed. All measurements were performed under the conditions described by Nicoletti et al. [11].

2.5. Cell counts

Cell proliferation was determined by counting the resulting cell numbers with a hemocytometer, using 0.4% trypan blue solution.

2.6. Statistical analysis

Cell count and flow cytometry data were based on four independent experiments with triplicate samples for exposed and control cells each. The Wilk–Shapiro rankit-plot test was used to assess the normal distribution of the data. Additional statistical analyses were made with the Student’s \( t \)-test. Differences between exposed and control cells with an error probability of \( p < 0.05 \) were considered to be statistically significant.

3. Results

3.1. Cell cycle distribution

Cell cycle distribution of U-937 and HCA-2/1\textsuperscript{1ch} cells from the groups exposed to EMF (25 Hz, 1.5 mT, 2 h and 45 min) were similar to the controls. The presence of Dex (0.3 µg/ml) during exposition and post-incubation 24-h period did not cause significant alterations of the cell cycle phases in relation to their controls. The percentage of control of each cell cycle phase from U-937 and HCA-2/1\textsuperscript{1ch} cells exposed to EMF with and without Dex are shown in Table 1. No significative differences have been observed in the results, \( p > 0.05 \) Student’s \( t \)-test.
Table 1

<table>
<thead>
<tr>
<th>Cell type and dexamethasone absence/presence</th>
<th>G0/G1 phase</th>
<th>S phase</th>
<th>G2 + M phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-937 -Dex</td>
<td>106.45 ± 12.15</td>
<td>94.92 ± 12.21</td>
<td>120.88 ± 34.36</td>
</tr>
<tr>
<td>+Dex</td>
<td>97.72 ± 2.3</td>
<td>105.46 ± 6.38</td>
<td>89.36 ± 13.24</td>
</tr>
<tr>
<td>HCA-2/1\text{tch} -Dex</td>
<td>98.46 ± 1.88</td>
<td>106.11 ± 1.21</td>
<td>83.82 ± 4.85</td>
</tr>
<tr>
<td>+Dex</td>
<td>110.05 ± 6.86</td>
<td>87.97 ± 7.66</td>
<td>104.24 ± 3.5</td>
</tr>
</tbody>
</table>

Mean values ± SD of four independent experiments in triplicate, (p > 0.05) Student’s t-test.

3.2. Apoptosis

The spontaneous apoptosis of U-937 and HCA-2/1\text{tch} cells exposed to EMF was similar to their matched controls (Fig. 1). Both cell lines from exposed and sham-exposed controls showed no significative differences of Dex-induced apoptosis (p > 0.05 Student’s t-test) (Fig. 1).

3.3. Cell proliferation

Twenty-four hours subsequent to the exposure in the magnetic field, the relative cell number (% of control) was observed to decrease to 55.84 ± 7.35% (p < 0.05 Student’s t-test) for HCA-2/1\text{tch} cells that were treated with dexamethasone and EMF exposure (Fig. 2). In contrast, no statistically significant changes were seen in the relative cell number neither for HCA-2/1\text{tch} exposed to EMF without Dex nor U-937 cells with and without Dex (Fig. 2).

4. Discussion

To study whether the EMF is acting as an inductor of apoptosis or a cell cycle redistribution agent in vitro, we investigated the potential effect of 25 Hz, 1.5 mT electromagnetic field. The spontaneous and Dex-induced apoptosis, the percentage of cells in each cell cycle phase and the cell growth were assayed in the presence and absence of dexamethasone.

We observed no significant alterations in the frequency of spontaneous and Dex-induced apoptosis in U-937 and HCA-2/1\text{tch} cells exposed to a rectangular and variable 25 Hz EMF at 1.5 mT field intensity. Besides, no alterations were observed in cell cycle distribution after exposure to EMF when compared with sham-exposed cultures.

These results complement previous reports showing negative cytotoxic results [12–14] reporting that EMF does not induce chromosome damage in vitro. Rosenthal and Obe [15] reported no spontaneous chromosomal effects in human lymphocytes, whereas pretreated cells showed an enhanced cell cycle progression. Antonopoulos et al. [16] described a stimulation of the cell cycle after exposure to 5 mT, 50 Hz EMF. A statistically significant increase of induction of apoptosis in a human squamous cell carcinoma cell line has been observed by Simkó et al. [4] after exposure to 0.8–1.0 mT EMF. Dex-induced apoptosis but not spontaneous apoptosis have been reported to be substantially increased in thymocytes exposed in vivo from 0.4 to 1.0 μT, 60 Hz EMF; nevertheless, spleen cells have not been shown to be affected by this field intensity [5].

Our experimental data are in disagreement with these
results because no alterations of apoptosis or cell cycle distribution could be observed in U-937 and HCA-2/1^ch cells.

On the other hand, a statistically significant decrease of the relative cell number (measured as % of sham-exposed controls) of HCA-2/1^ch cells after EMF exposure in the presence of dexamethasone, but not in the absence, was observed. No effect, however, was found for U-937 cells. These findings suggest that EMF in the presence of dexamethasone could probably produce an alteration in cell growth that could be different for different cell lines.

In a previous paper [7], we have reported no alterations in cell growth of a human colon adenocarcinoma cell line with a similar exposure period to 25 Hz, 1.5 mT EMF.

Hisamitsu et al. [2] and Narita et al. [1] have observed a decrease in cell viability with exposure times of 0.5–3.5 h, for HL-60 and ML-1 cells exposed to a 45-mT, 50-Hz magnetic field.

These results suggest that 25 Hz, 1.5 mT EMF do not induce apoptosis or cell cycle alterations in U-937 and HCA-2/1^ch cells when they are exposed for 2 h and 45 min. On the other hand, the presence of dexamethasone during the exposition to EMF could probably produce a decrease in cell growth in HCA-2/1^ch cells. Finally, this study represents a new approach to investigating the effects of ELF-EMF exposure on tumoral cell biology.

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References