

Induction of chromosomal aberrations in human primary fibroblasts and immortalized cancer cells exposed to extremely-low-frequency electromagnetic fields

S.S. Seyyedi¹, H. Mozdarani^{2*}, M. Rezaei Tavirani^{1,3}, S. Heydari¹

¹Proteomic Research Center, Shahid Beheshti University, Tehran, Iran

²Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

³Faculty of Paramedical Sciences, Shahid Beheshti University, Tehran, Iran

Background: Rapidly increasing possibilities of exposure to environmental extremely low-frequency electromagnetic fields (ELF-EMF) have become a topic of worldwide investigation. Epidemiological and laboratory studies suggest that exposure to ELF-EMF may increase cancer risk therefore assessment of chromosomal damage in various cell lines might be of predictive value for future risk estimation. **Materials and Methods:** Primary cultures of fibroblasts from human skin biopsy were exposed to continuous extremely low-frequency electromagnetic fields (3, 50 and 60 Hz, sinusoidal, 3h, and 4 mT). Also immortalized cell lines, SW480, MCF-7 and 1321N1 were exposed to continuous ELF-EMF (50 Hz, sinusoidal, 3 h, 4 mT). Metaphase plates were prepared according to standard methods and stained in 5% Giemsa solution. Chromosomal aberrations of both chromosome and chromatid types were scored to evaluate the effects of ELF-EMF on primary or established cell lines. **Results:** Results indicate that by increasing the frequency of ELF-EMF, chromosomal aberrations were increased up to 7-fold above background levels in primary human fibroblast cells. In addition, continuous exposure to a 50 Hz electromagnetic field led to a significant increase in chromosomal aberrations in SW480, MCF-7 and 1321N1 cell lines compared to sham control. **Conclusion:** Results obtained indicate that ELF-EMF has the potential for induction of chromosomal aberrations in all cell types. *Iran. J. Radiat. Res.*, 2010; 8 (1): 25-29

Keywords: ELF-EMF, chromosomal aberrations, continuous exposure, human fibroblasts, cancer cell lines.

INTRODUCTION

Nowadays, there are many electronic devices that generate electromagnetic fields, such as household appliances as televisions, electric blankets, hair driers, and therapeutic medical applications^(1, 2). The widespread

use of electric power and higher levels of EMFs encountered in the environment have raised concerns that such exposures might be associated with cancer risks^(3, 4). The first suggestion of possible health hazards of environmental exposure to ELF-EMF were derived from studies performed in the former USSR in the early 1960s⁽⁵⁾.

During the last few years, several comprehensive investigations have been performed in laboratories world-wide to assess the biological effects of ELF magnetic and electric fields^(6, 7). However, the result obtained in various researches is controversial^(6, 8).

Several epidemiological studies have indicated ELF-EMFs increased risk of cancer, especially acute childhood leukemia and cancer of the central nervous system⁽⁹⁻¹³⁾ and revealed symptoms of nervous system dysfunction (e.g. vegetative dystonia, neurasthenic/hypertonic symptoms) or cardiovascular disorders (e.g. arrhythmia, increased heart rate and arterial pressure) during and shortly after ELF-EMF exposure^(14, 15).

In parallel with epidemiological studies, several *in vivo* and *in vitro* models have been investigated in laboratories to whether a link existed between ELF-EMFs and potential adverse biological effects (e.g.

*Corresponding author:

Prof. Hossein Mozdarani;

Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, P.O. Box: 14115-111, Iran.

Fax: +98 21 88006544

E-mail: mozdarah@modares.ac.ir

mutagenesis and carcinogenesis), and to determine the possible mechanism of cancer induction⁽¹⁶⁻²⁰⁾.

However, it is well accepted from the totality of the available evidence that such fields do not possess sufficient energy to generate direct DNA damage. Several contradicting result have been obtained for cells of different species and tissues using the genotoxic endpoints such as DNA strand breaks, chromosome aberrations (CA) micronuclei (MN) formation and sister chromatid exchange (SCE), after exposure to ELF-EMF at various exposure conditions⁽²¹⁻²³⁾. The majority of these studies did not show any ELF-EMF related genotoxic effects. But some investigations revealed that exposure of human cells to ELF-EMF cause significant increases in micronuclei and chromosomal aberrations^(24, 25).

Despite the large number of studies performed, the effect of ELF-EMF on genotoxicity is still unclear⁽²⁶⁾. In the present study, the possible genotoxic effects of ELF-EMF, on human fibroblast cells derived from skin biopsy and established cell lines were studied using chromosomal aberrations as biological end points.

MATERIALS AND METHODS

Cell culture

Human fibroblasts cell culture was initiated from skin biopsy and in Dulbecco's modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal calf serum, 2mM glutamine and antibiotics [penicillin (100 u /ml) and streptomycin (100 µg/ml)]. Cells were maintained in 75 cm² flasks and left at 37 °C in a CO₂ (5 %) incubator.

MCF-7 cells were obtained from national cell bank of Iran (NCBI, number: C135). This cell line was established from pleural effusion obtained from a 69-year-old Caucasian female. Cells exhibit some features of differentiated mammary epithelium. The morphology of the cells is epithelial-like.

SW480 cell line was also obtained from NCBI (number: C506). This cell line was established from a colon adenocarcinoma obtained from a 51-year-old Caucasian male. The cells produce carcinoembryonic antigen (CEA), keratin, transforming growth factor beta and GM-CSF and express receptors for epidermal growth factor (EGF). The morphology of the cells is epithelial-like.

1321N1 cells were obtained from NCBI (number: C118). This cell line was derived from a human brain astrocytoma. The morphology of the cell is glial-like.

SW480, MCF-7 and 1321N1 cell lines were maintained in RPMI-1640 supplemented with 10% fetal calf serum (FCS), 2mM glutamine, penicillin (100 u /ml) and streptomycin (100 µg/ml) at 37 °C in an incubator containing 5 % CO₂.

All of the cells were passaged twice weekly and used for experiments while exponentially growing. For experiments, cells were cultured in 75 cm² flasks set up at 3 × 10⁵ cells per flask in 10 ml of RPMI-1640 for cancer cell lines and DMEM for skin fibroblast cells and grown for 2 days.

ELF-EMF exposure conditions

EMF exposure systems produced a homogenous vertical ELF-EMF (sinusoidal, 3, 50 and 60 Hz, 4mT) generated by a solenoid. Cells were exposed to EMF for 3 hours. Temperature was continuously monitored. The temperature difference did not exceed 0.3 °C throughout the experiments. Control cells were sham exposed in the same exposure chamber but the system was switched-off.

Chromosomal aberrations

After ELF-EMF exposure, cells were incubated at 37 °C for 12 hours then treated with colcemid at a final concentration of 0.4 µg/ml for 4 h to arrest cells in mitosis. Cells were then harvested using 0.05% trypsin treatment for 5 min. Cells were centrifuged, medium removed and re-suspended in 10 ml

hypotonic solution (0.075 M KCl) kept at room temperature for 15 min. Cells were fixed in cold Carnoy's fixative (methanol:acetic acid, 3:1). The fixation procedure was repeated three times before spreading on slides and staining with Giemsa (5% in Gurr's buffer pH 6.8 for 5 min). Metaphase spreads were scored under a Zeiss light microscope with a magnification of $\times 1000$.

Two hundred metaphases per sample were scored for the number of chromatid and chromosomal aberrations. Different types of chromatid and chromosome aberrations; including gaps, isogap, breaks, deletion, chromatid and chromosome exchange and double minutes were separately scored.

Statistical analysis

Statistical analysis was carried out using the SPSS statistical software (version 16.0). One-way analysis of variance (ANOVA) was performed to evaluate differences between exposed and sham-exposed, as well as between different exposure conditions. A difference at a level of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Results are summarized in table 1 and shown in figure 1. As seen the frequency of chromosomal aberrations in normal primary cells derived from skin biopsy and exposed to 3, 50 and 60 Hz EMF, increased as the intensity of EMF field increased. Considering total aberrations, significant differences ($p < 0.05$) between exposed and sham-exposed cells were observed. Also the difference between results obtained for cells exposed to 3 Hz and 50 or 60 Hz EMF was statistically significant ($p < 0.05$). These findings showed an increase of nearly 7-fold for total aberrations for cells exposed for 3 h to 50 and 60 Hz electromagnetic field compared to sham exposed cells (figure 1).

Table 1 also show the results obtained for cell lines used in this study and exposed for 3 hours continuous 50 Hz electromagnetic field. Frequency of aberrant cells and chromosomal aberrations in MCF-7, SW480 and 1321N1 cell lines increased dramatically compared to sham exposed control (table 1). Data obtained shows statistically significant difference ($p < 0.05$) between

Table 1. Number of chromosome and chromatid type aberrations induced by ELF-EMF exposure in cultured normal human fibroblasts, MCF7, SW480 and 1321 cell lines.

Cell line	Treatment	Chromosome aberrations					Chromatide Aberration				Total	Aberrant Cells
		Iso. Gap	Ch. Break	Ch. Exchange	Iso. Ch. Break	Double Minute	Gap	Ctd. Del.	Ctd. Exchange	Iso. Ctd. Del.		
Sham Exposed	--	--	--	--	--	--	--	3	--	--	3	3
Human Fibroblasts	3 Hz	--	--	--	3	2	--	7	--	--	12	7
	50 Hz	2	2	4	1	--	2	11	1	--	23	10
	60 Hz	1	3	3	2	3	--	12	1	1	26	11
1321N1	50 Hz	--	4	2	--	3	2	16	--	--	27	13
SW480	50 Hz	--	2	3	1	3	3	13	--	--	25	11
MCF7	50 Hz	--	5	3	4	2	3	11	--	2	30	10

exposed and sham-exposed cells (figure 1). However, there was no statistical significant difference between results obtained for different cell lines, although MCF-7 cell line showed more sensitivity to EMF field compared to other cell lines.

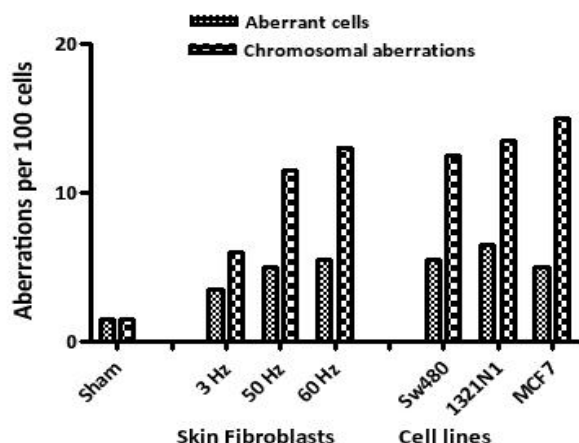


Figure 1. Percentage of total aberrant cells and total chromosomal aberrations induced by ELF-EMF exposure in cultured normal human fibroblasts, MCF7, SW480 and 1321N1 cell lines.

Results indicate that continuous exposure of normal human fibroblast cells led to an increase in the frequency of different types of chromosomal aberrations as the frequency of EMF increased (table 1). Therefore, the extent of damage depends on the frequency of exposure. These findings are in agreement with some studies with continuous ELF-EMF exposure of the cells which also have shown an increase in chromosomal damage (25-27). In a study Ivancsits *et al.* (28) have shown that biological effect of ELF-EMF was cell type specific. Based on their results, some cell types such as human fibroblasts, human melanocytes, transformed rat granulosa cells were responder and cell types such as human lymphocytes, monocytes and skeletal muscle cells were non-responder to ELF-EMF (28).

Our findings shown in table 1 and figure 1 are different with the report of Ivancsits *et al.* (28). As seen in figure 1 relatively similar frequency of chromosomal aberrations are induced by 50 Hz ELF-EMF

in human primary fibroblast cells and three cell lines SW480, 1310N1 and MCF-7 with different origins. SW480 cell is colon adenocarcinoma and MCF-7 is breast cancer. But 1321N1 is human brain astrocytoma cell. Therefore, all of these cells with different origins have shown similar reaction to ELF-EMF exposure.

The fate of a cell carrying a chromosomal aberration is crucial for the assessment of a possible cancer risk. Cells with unstable aberrations of either chromosome or chromatid type might be committed to apoptosis or cell death, whereas cells with balanced rearrangements and repairable chromosomal damages like gaps, isogaps or simple breaks may survive. Misrepair or partially repaired damages might lead to induction of stable mutations involved in carcinogenesis (27, 29, 30). Although amongst various types of DNA damages involved in the production of chromosomal aberration, the nature of DNA damage induced by ELF-EMF is not well defined, but DNA fragmentation due to ELF-EMF (23) might be involved in the induction of chromosomal aberrations following exposure to ELF-EMF.

In conclusion, results indicate that induction of chromosomal aberrations was dependent on the frequency of ELF-EMF and similar effect was seen in different cell types studied after exposure to ELF-EMF.

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