THE CONSEQUENCES OF LOW FREQUENCY AND INTENSITY ELECTROMAGNETIC FIELDS ON THE FREQUENCY OF MICRONUCLEI IN HeLa CELLS

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Abstract: The treatment of HeLa neoplastic cells with low frequency and intensity electromagnetic field has determined modifications of the micronuclei number, this impact being correlated with the application manner of the electromagnetic field (continuous or discontinuous). Thus, the continuous electromagnetic field has reduced the frequency of the micronuclei formation (2.91 ± 0.015 ‰), as compared to the value of control group (3.93 ± 0.023 ‰), while the discontinuously applied electromagnetic field has increased the number of micronuclei (4.92± 0.012 ‰). These variations in micronuclei number suggested that low frequency electromagnetic field interfere in different ways with the genetic material of cancerous cells, indicating that the cEMF had a protective effect upon DNA molecule, while dcEMF had a genotoxic impact. Also, the estimation of the micronuclei area has revealed that the area of micronuclei generated by dcEMF was smaller than that of cEMF.

1 INTRODUCTION

Low frequency and intensity electromagnetic fields are a part of our life due to the large scale utilization of computers, home appliances, radio communications or of the other electrical devices. Now, everyone is living in a mix of weak electric and magnetic fields, the impact upon our organism being still under investigation. The majority of studies have investigated the possible negative effects of low frequency and intensity electromagnetic fields upon humans (Hardell & Sage, 2008; Heynick, Johnston & Mason, 2003; Johansson, 2009; Kavet, 1996; Verschaeve *et al.*, 2006).

A few studies have been oriented towards the investigation of the impact of the electromagnetic fields upon the cancerous cells and the evaluation of consequences of the exposure on this type of cells to the low frequency and intensity EMF (Falone *et al.*, 2007; Girgert, Gründker, Emons & Hanf, 2008; Ronchetto *et al.*, 2004; Tenuzzo *et al.*, 2006). The findings obtained on this experimental model are contradictory and full of gaps due to the lack of an unitary exposure setup, protocol or used biological material.

The necessity to understand and investigate the possible functional interactions between electromagnetic fields and cancerous cells is determined by the fact that electromagnetic fields are incriminated to facilitate the carcinogenicity (Juutilainen & Lang, 1997; Juutilainen, Kumlin & Naarala, 2006; Mairs *et al.*, 2007; Meltz, 2003; Simkó, Kriehuber, Weiss & Luben, 1998; Thun-Battersby, Mevissen & Löscher, 1999). From this point of view is important to identify the most dangerous characteristics (frequency, intensity, amplitude modulations, time of exposure) of electromagnetic fields to limit the influence upon healthy or tumor bearing persons. One of the ways to estimate the impact of electromagnetic fields upon cells is represented by quantification of micronuclei occurrence in the exposed cells.

The micronuclei (MN) test is the most frequent technique used to detect chromosome breakage or mitotic interference of different xenobiotics, events thought to be associated with increased risk for cancer or with a supplementary genetic destabilization of already mutated cells (like cancerous ones) (Wolff & Muller, 2005).

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This work presents an initial investigation about the impact of the low frequency and intensity electromagnetic field upon the integrity of HeLa's genetic material by registration of the variations in the frequency of the micronuclei occurrence.

2 MATERIAL AND METODS

The biological material used in the *in vitro* experiments was represented by mycoplasm-negative HeLa cellular cultures of human neoplastic origin.

HeLa cells were cultured in DMEM medium (Dulbeco's Modified Eagle's Medium, Biochrom AG, Germany, FG 0415), supplemented with 10.0% fetal bovine serum (Sigma, Germany, F9665), 100 µg/mL streptomycin (Biochrom AG, Germany,A 331-26), 100 IU/mL penicillin (Biochrom AG, Germany, A 321-44) and 50 µg/mL antimycotic amphotericin B (Biochrom AG, Germany, A 2612), at a density of 5 x 10^5 cells / 25 cm^2 flask, at 37^{o}C When the cells reached confluence in the monolayer stage, the cultures were divided into control and electromagnetic treated cell cultures.

The electromagnetic field (EMF) of continuous or discontinuous type (cEMF, dcEMF) was generated by an IBF magnetodiaflux device. This presents two circular coils (29 cm in diameter, placed at a distance of 14.5 cm) disposed on a cardboard cylinder, which delimits the place where the culture flasks are maintained during the electromagnetic treatment. The intensity and frequency of the generated electromagnetic field were of 5.5 mT and 100 Hz.

Single EMF was applied continuously or discontinuously (with breaks of 1 second and action 3 seconds) to the cell cultures, for a period up to 60 minutes. Simultaneous experiments skipping the electromagnetic field were also performed on the control cultures. During the real or blind treatment the cell cultures were removed from the incubator and placed into magnetodiaflux, where the temperature has reached up to 30^{0}C.

After the electromagnetic treatment, the cell cultures were left for another 24 hours in incubator and then were subjected to quantification of the micronuclei number. First, the medium was discarded, the cell layer was rinsed with PBS (phosphate buffer saline) and than was detached from the surface of the flasks with a solution of trypsin (Biochrom AG, Germany).

The cell suspension was resuspended in normal growth medium and centrifuged at 1800 rpm for 2 minutes. Over the pellet was added fresh and cooled Carnoy's fixative. The slides preparation was made by air-dry method and then observed at a Nikon Eclipse 600 microscope, at the magnification of 4000x.

The frequency of micronuclei was calculated as the number of micronuclei at 1000 read interphases. The determination of the micronuclei area was realized with ImageJ software and the calculation of the approximate surface of micronuclei was based on the circle area formula.

The results are expressed as mean ± SE and were statistically analyzed by Students't' test. The p value <0.05 was considered significant.

3 RESULTS AND DISCUSSIONS

The evaluation of the consequences determined by the exposure of HeLa cells to the continuously or discontinuously applied electromagnetic fields upon the genetic material integrity was based on the estimation of the micronuclei occurrence frequency to 1000 interphases, as compared to the specific MN frequency of the control group (Figure 1). The micronuclei occurrence frequency in the control group was of 3.93 ± 0.023 ‰, value which was considered by us as reference and was equated to 100%.

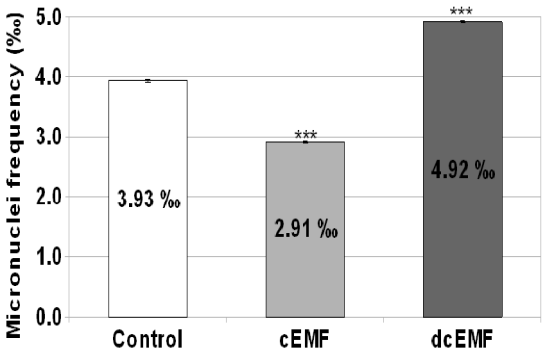

Figure 1: Micronuclei occurrence frequency (expressed as procentual values) in the case of the HeLa neoplastic cell cultures untreated or treated with continuous or discontinuous electromagnetic field (100 Hz, 5.5 mT) for 60 minutes. Significantly different from control: *p<0.05, **p<0.01, ***p<0.001.

As compared to the control group, the electromagnetic treatment applied continuously has determined a reduction of the micronuclei frequency to the value of 2.91 ± 0.015 ‰, corresponding to a procentual depletion of 25.84%.

Contrary, the discontinuous electromagnetic field has induced an augmentation of the micronuclei frequency, the registered value (4.92± 0.012 ‰) being with 25.20% over the reference.

Another approach of the research was the estimation of the micronuclei total area, determined using photos, as an attempt to quantify the amount of genetic material expelled with micronuclei (Table 1).

Table 1: Mean micronuclei area (μm^2) and procentual variations specific to the control group and to the treated groups either with cEMF or with dcEMF.

Experimental group	Micronuclei total area (μm^2)	% variation
Control	13.01	100
cEMF	50.10	385.09
dcEMF	20.59	158.26

The mean area of the micronuclei of the control group was of 13.01 μm^2, while the groups treated with cEMF and dcEMF, respectively, have presented a mean area of 50.10 μm^2 and 20.59 $\mu m^{2,}$ respectively.

Ionizing radiations along with other numerous chemical mutagens causes structural chromosomal aberrations, some of them being visible at the light-microscope level. The aberrations from the level of chromosome can generate chromosome fragments without spindle attachment organelles, being called acentric fragments. When the cell divides, some of these fragments are excluded from the main daughter nuclei and form small extra nuclei within the cytoplasm, either on their own, or in conjunction with other fragments. Such "micronuclei" (MN) can appear in the cytoplasm of either, or both, daughter cells (Savage, 2000)

The electromagnetic field treatment, continuously or discontinuously applied, has determined, as compared to the reference value, fluctuations in the micronuclei occurrence frequency. These effects, specific for every type of electromagnetic field, are due to probably different patterns of interaction between EMFs and the genetic material of the HeLa cells.

Our presumption regarding the reduced frequency of micronuclei in the experimental group treated with cEMF is that cEMF either modifies the electric charge of DNA molecule, followed by a stabilization of the genetic material, or intensifies the activity of molecular mechanisms responsible for maintaining the DNA integrity. Also, changes in the electrical charge of DNA macromolecule could explain the higher area of micronuclei, as compared to control group and dcEMF. In this case, the breakage of the DNA structure could occur in the zones where the fragility of DNA is higher, generating fragments of genetic material.

The elevated number of micronuclei in cells treated with dcEMF suggests the weakening of the DNA integrity either by induction by dcEMF of microoscilations in the structure of DNA, with the generation of suplimentary breakages, or by modification of the intracellular microenvironoment constants, with brutal and destructive consequences upon genetic material integrity (Davies *et al.*, 1999). As in the case of cEMF, the area of micronuclei after dcEMF action was higher than that of the reference group, but smaller than of cEMF, suggesting the release of shorter DNA fragments.

From the above presented data, we can conclude the existence of an inverse relationship between frequency of micronuclei and their area, suggesting different sites of interaction of EMF with DNA, with immediate consequences upon its integrity.

Our experimental data, obtained in this experimental frame, are overlapping with those from the specialty literature (that contains limited references to cancerous cells) - which has signaled the sporadic existence (Hee Cho & Chung Won, 2003; Juutilainen, Heikkinen, Soikkeli & Mäki-Paakkanen, 2007) or even absence (Pasquini *et al.*, 2003; Speit, Schütz & Hoffmann, 2007; Verschaeve *et al.*, 2006) of some genotoxic effects induced by electromagnetic fields, generator of micronuclei.

4 CONCLUSIONS

The EMF interaction with neoplastic HeLa cells has generated an increase (dcEMF) or a decrease (cEMF) of the micronuclei occurrence frequency, suggesting different sites and ways of action upon the genetic material of cancerous cells.

cEMF expressed a protector effect upon genetic integrity of HeLa cells, while the dcEMF had a genotoxic impact upon DNA molecule.

Software analysis of the expelled micronuclei area has showed that EMF has generated micronuclei with higher areas than those in the case of the control group and allowed the establishment of an inverse relationship between micronuclei occurrence frequency and their areas.

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