Study of Electromagnetic Fields on Cellular Systems

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ABSTRACT

In the last decades the interest to study the effect of non-ionizing radiation, such as the electromagnetic fields (EMF) on cellular systems has increased. In this article the interaction between EMF and biological systems is described. An analysis of the effect of the electromagnetic stimulation at different frequencies and intensities on cell cultures is performed. Preliminary results show that the stimulation with extremely low frequency electromagnetic fields (ELF-EMF), EMF from 3 to 30 Hz, on the cellular line of neuroblastoma SK-NSH induces cellular stress. This is reflected by a variation in the proteins expression in comparison with the group of cells no stimulated. In particular, the proteins expression shows that the ELF-EMF produce changes in the current proteins in normal or basal conditions in the cells, that is, new proteins appear or there is evidence of an increasing in the amount of them.

RESUMEN

Durante las últimas décadas, el interés por explicar el efecto de la radiación no ionizante, como es el caso de los campos electromagnéticos (CEM) sobre sistemas celulares ha aumentado considerablemente. En este artículo se describe la interacción que existe entre los CEM y sistemas biológicos. Se discute el efecto de la estimulación electromagnética a diferentes frecuencias e intensidades en cultivos celulares. Resultados preliminares al estimular células de neuroblastomas SK-NSH con campos electromagnéticos de extra baja frecuencia (CEM-EBF), CEM que van del rango de 3 a 30 Hz, indican que se induce un estrés celular que se refleja en variaciones en la expresión de proteínas respecto al grupo de células no estimuladas. En particular, la expresión de las proteínas muestra que los CEM-EBF producen cambios en las proteínas presentes en condiciones normales o basales en las células, es decir, aparecen nuevas proteínas o existe un aumento en la cantidad de ellas.

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INTRODUCTION

The number of cells in the human body is literally astronomical, about three orders of magnitude greater than the number of stars in the Milky Way. Yet, for their immense number of cells in the body, the variety of cells is much smaller: about 200 different cell types are represented in the collection of about 10^{14} cells that make up our bodies (Boal, 2002, p. 17).

Keywords:
Electromagnetic stimulation; Extremely low frequency electromagnetic field; Magnetobiology; Neuroblastoma.

Palabras clave:
Estimulación electromagnética; Campos electromagnéticos de extra baja frecuencia; Magnetobiología; Neuroblastomas.

With the great technological advances made in recent years, the use of the EMF has been more common in different areas; such as medicine, communication, industry, science, etc. In the medicine field has been developed many devices that utilized MF, especially Magnetic Resonance Imaging (MRI) devices. In radio communications, the use of EMF has been useful in the design of a new kind of mobile phones (James *et al.*, 1982; Linde and Mild, 1997).

In recent years, the interest to study the possible effects of the EMF on biological systems has increased. This has promoted a new area of study called Magnetobiology. This field studies the effect of the EMF on biologi-

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cal systems, such as human being, animals, and plants. Due to the fact that human beings are in constant exposure to different magnetic and electromagnetic sources, there is an increased interest in studying the possible effects of EMF such as high voltage electric power lines (Zhadin, 2001; Grunder *et al.*, 1991). Furthermore, the numbers of studies about the exposure to radiofrequency EMF have increased considerably due to interest in the possible effects of the growing use of cellular phones (Challis, 2005).

Nevertheless, the effects that EMF has on cellular systems are not well understood. Several studies have reported that different physical parameters such as wavelength, the way of the wave, frequency, and intensity affect the stimulation capacity, as well as the kinds of cells exposed. Most of these studies have taken an interest in describing the effect of EMF at 50 – 60 Hz on cells due to a cancer incidence at that frequencies range (Tenforde, 1992). Other reports show that EMF are useful in therapy, e.g., in wounds and bone fractures (Funk, 2006).

The aim of this article is to give a description of the interaction between EMF and cellular systems. Additionally, this article aims to show some of the preliminary results of the effects of ELF-EMF, that is EMF with frequencies from 3 to 30 Hz, on a cellular line of neuroblastoma.

RADIO WAVES AND THE INTERACTION OF EMF WITH MATTER

The electromagnetic spectrum is the range of all possible electromagnetic radiation frequencies, both ionizing and non-ionizing. However, this work focuses on radio waves, which are non-ionizing radiation, a form of electromagnetic radiation that does not carry enough energy to remove an electron from an atom or molecule. The radio waves are electromagnetic waves (EMW) that take place on the radio frequency region of the electromagnetic spectrum. The radio waves are divided in different frequency ranges, see table 1.

Table 1.
Frequencies range of the radio waves.

Name	Frequency Range	Wavelenght
	Lower than 3 Hz	> 100.000 km
Extremely low frequency (ELF)	3-30 Hz	100.000 km – 10.000 km
Super low frequency (SLF)	30-300 Hz	10.000 km – 1000 km
Ultra low frequency (ULF)	300–3000 Hz	1000 km – 100 km
Very low frequency (VLF)	3–30 kHz	100 km – 10 km
Low frequency (LF)	30–300 kHz	10 km – 1 km
Medium frequency (MF)	300–3000 kHz	1 km – 100 m
High frequency (HF)	3–30 MHz	100 m – 10 m
Very high frequency (VHF)	30–300 MHz	10 m – 1 m
Ultra high frequency (UHF)	300–3000 MHz	1 m – 100 mm
Super high frequency (SHF)	3-30 GHz	100 mm – 10 mm
Extremely high frequency (EHF)	30-300 GHz	10 mm – 1 mm
	Higher than 300 GHz	< 1 mm

In general, EMW are a form of radiation or energy that can be propagated through vacuum or matter. They travel in vacuum with a speed of 2.998×10^8 meters / second. In other mediums, the propagation of EMW would depend on the transport characteristics of the matter. The behaviour of EMW can be described by a wave or by particle-like bundles of energy that are called quanta or photons (Halliday *et al.*, 1999, p. 311 - 324; Beiser, 2003, p. 53).

EMW can decompose into two mutually dependent perpendicular components. The first component carries the electrical properties and is therefore called Electrical Field (EF). The other component exhibits the magnetic properties of the radiation and is therefore known as Magnetic Field. Both components propagate in space with the same frequency, speed and phase. Therefore, the EMF are EMW with an electric and magnetic component, see figure 1.

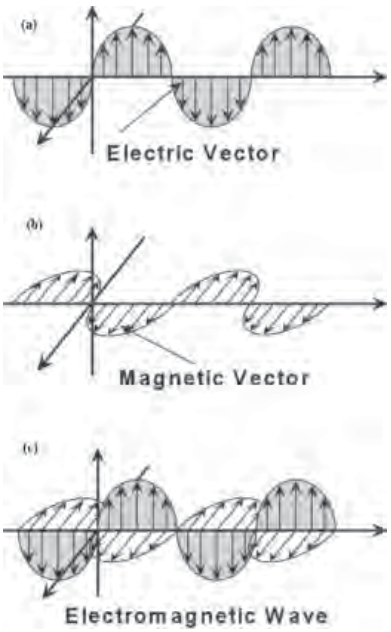

Figure 1. (a) and (b) the electric and the magnetic component of a electromagnetic wave, are shown respectively. (c) The electromagnetic wave is composed of an electric and a magnetic component. These components are perpendicular to each other and to the direction of propagation of the wave.

On the other hand, the interaction of the EMF with biological systems must not be a result of the influence of a foreign energy, as in the case of the ionizing radiation, it must be considered as a modification of the proper electric and magnetic structure of the biological system.

When the mechanisms of the interaction between matter and energy are observed, three questions should be analyzed:

1) How does the applied EMF enter the body?

2) How does the introduced EMF interact with the biological system, molecules, and cells?

3) What are the biological consequences of the primary effects produced?

The first problem is purely a matter of physics. It can be solved by using the Maxwell equations and knowing the impedance of the body and the corresponding issue, see equations 1- 4.

$$\nabla \bullet \mathbf{D} = \rho_f, \quad (1)$$

$$\nabla \times \mathbf{E} = -\frac{\partial \mathbf{B}}{\partial t}, \quad (2)$$

$$\nabla \bullet \mathbf{B} = 0, \quad (3)$$

$$\nabla \times \mathbf{H} = \mathbf{J}_f + \frac{\partial \mathbf{D}}{\partial t}, \quad (4)$$

where **E** and **B** are the electric and magnetic field respectively, **D** the electric displacement and **H** the magnetic field intensity. Whereas, ρ_f and $\mathbf{J}_f$ are the free charge density and free current density respectively.

The second question poses a truly biophysical problem. In answering this question, one must accept the electrical structure of the biological system *in vivo* (Graser, 1992). From the perspective of physics, it is possible to study the biological systems by using different physical laws of various theoretical approaches, see table 2.

Table 2.
Physical approaches to study biological systems.

Biological Structure	Point of View
Organism Level	Electrodynamics (Maxwell Equations)
Cellular Level	Thermodynamics (Nerst-Plank)
Molecular Level	Statical Thermodynamics (Poisson – Boltzmann)
Atomar Level	Quantum Mechanics (Schrödinger Equation)

MAGNETIC AND ELECTROMAGNETIC STIMULATION

The magnetic stimulation must be associated only with a MF. The easiest way to produce a MF is with a magnet. However, if an inductive exposure system such as a coil is used (Helmholtz coil, solenoids, etc.), a MF is produced since according to the Biot-Savart Law is known that a stationary electric current in a wire produces a MF (**B**) around it. In this case such field is static, and no EF will be produced. On the other hand, the electromagnetic stimulation is linked with both an electric and magnetic field. When the MF varies with time, an induced EF will be produced according to Faraday's law of induction. The EF is proportional to the time variation of the MF ($\partial\mathbf{B}/\partial t$) and an EMF is produced.

The electromagnetic stimulation can be performed by using different systems. There are different types of exposure devices. Amongst the most commons ones are solenoids, Helmholtz coils, etc. The Helmholtz coils are useful because of generates a homogeneous MF. The MF can be classified according to its intensity in this manner; weak (< 1 mT), moderate (from 1 mT to 1T), strong (1 – 5 T) and ultra- strong (> 5T). The MF can be classified by its frequency as described in the table 1.

Furthermore, another important characteristic of the electromagnetic stimulation is the shape of the signal because of is thought that the use of pulsed or modulated fields yields an additional problem. Many investigators and therapists use frequencies of 150 or 450 MHz, either modulated or pulsed, with frequency ranges from 10 to 20 Hz (Dutta and Millis, 1986). This treatment is frequently followed because it is believed that a high frequency penetrates the biological material. However, the low frequency is in fact the frequency component which acts to penetrate the biological system. The signal shapes can be sinusoidal, square, sawtooth, triangular, etc. see figure 2.

ELECTROMAGNETIC STIMULATION OF NEUROBLASTOMAS

With the proposal to apply EMF to cellular systems, the electromagnetic stimulation of neuroblastoma (one of the most common types of solid pediatric tumor that originate in the neural crest) was performed. Two groups of study were analyzed, cells exposed to electromagnetic stimulation (stimulated group) and cells with no exposure to electromagnetic stimulation (control group).

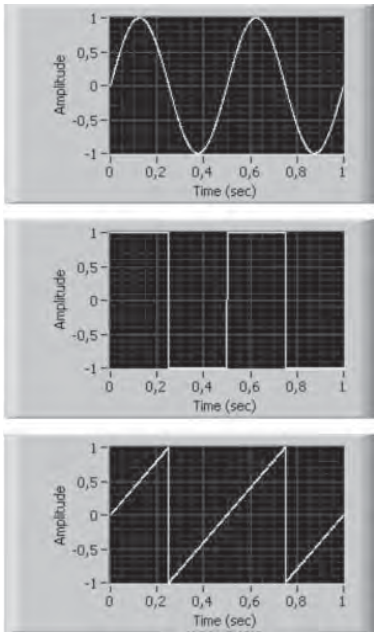

Figure 2. Various kinds of wave shapes; sinusoidal, square and sawtooth.

MATERIAL AND METHODS

Cell cultures

The human neuroblastoma cell line SK-NSH was grown (1 x 10^5 cells/mL) in DMEM "Dulbecco's Modified Eagle Media" supplemented with 10 % of fetal calf serum at 37 °C, and 5% of CO_2, after 6 days the cells were exposed to MF.

Exposure Conditions

SK – MNH cells were exposed for one hour to a MF of 19 mT. The exposure system was built for the engineering department of the Guanajuato University, and consisted of one Helmholtz coil. The cells were stimulated by square waves at the rate 8 Hz. Both the stimulated and control samples were maintained outside the incubator at room temperature. The stimulated sample was placed in the homogenous region of the Helmholtz coil and the MF was applied perpendicularly to the sample.

Electrophoresis Studies

After the time of stimulation following the standard protocol, both the stimulated and the control cell groups were prepared for carrying out gel electrophoresis studies. The samples were fractionated in 10% SDS-PAGE gel (sodium dodecyl sulfate polyacrylamide gel electrophoresis). The gels were charged with the extract of the proteins (25 – 30µL) to be analyzed. The samples were run in a Mini-Gel Systems vertical electrophoresis chamber (C.B.S Scientific Company, Inc MGU-402, USA) fed with a power supply (BIO- RAD) at 80 Volts during 2 hours. In order to evaluate whether the proteins are of high, medium or low molecular weight, in one lane molecular markers were used where as in the other lanes, the control and stimulated samples were placed respectively. In addition, the molecular weight of the peptides or molecules is determined for the relative mobility produced by the electric field. Whether a peptide is of high, medium, or low molecular weight is function of the velocity at which the particles travel in the medium as well as of the electric surface charge of the peptide.

DNA Extraction

Genomic DNA extraction was performed with TSNT technique (Casas *et al.*, 2008). The samples were analyzed in agarose gel at 1% with Ethidium Bromide. The agarose gel method separates molecules by size where the negatively charged nucleic acid molecules move through an agarose matrix with an electric field. The bands were visualized and photography in Gene Geniuses Syngene on a UV transilluminator at 260 nm and 280 nm.

RESULTS

Figure 3 shows the profiles of proteins in the human neuroblastoma cell in the control and stimulated groups. Comparing with molecular markers peptides of high, medium, and low molecular weight were visualized. The bands definition was similar for the two groups. No qualitative differences were evident between the two groups, so it was not possible to identify alterations in the synthesis of proteins only by using the electrophoresis gel.

In order to investigate in more detail whether MF has an effect on the basal expression of proteins, a quanti-

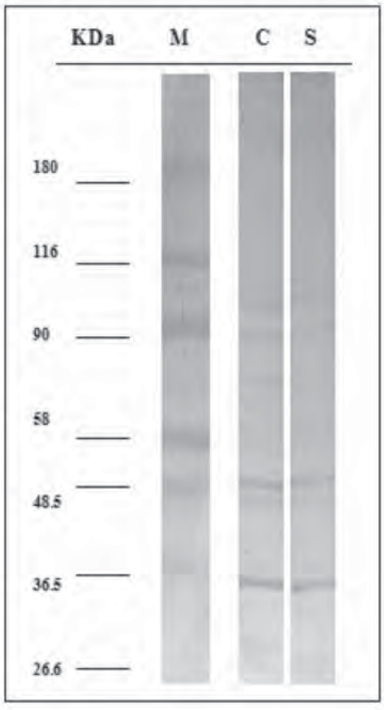

Figure 3. The proteins profile is shown. (C) and (S) represent the control and stimulated groups respectively, whereas (M) represents the molecular weight markers in Kilodaltons (KDa).

tative analysis was performed. In figure 4, a comparison between the stimulated and control group is shown. A representation of the percentage of proteins and molecular weight is shown. The stimulated group shows a higher concentration of proteins than the control group. Due to the fact that the area and intensity of the bands are related to the protein concentration, figure 4 was determined using the results of figure 3. The intensity and area of the bands of the figure 3 were analyzed with an image processing program built in MATLAB 8.0. This software was used to plot the molecular weight versus the protein percentage (intensity and area of the bands) to determine the possible variations produced by the MF.

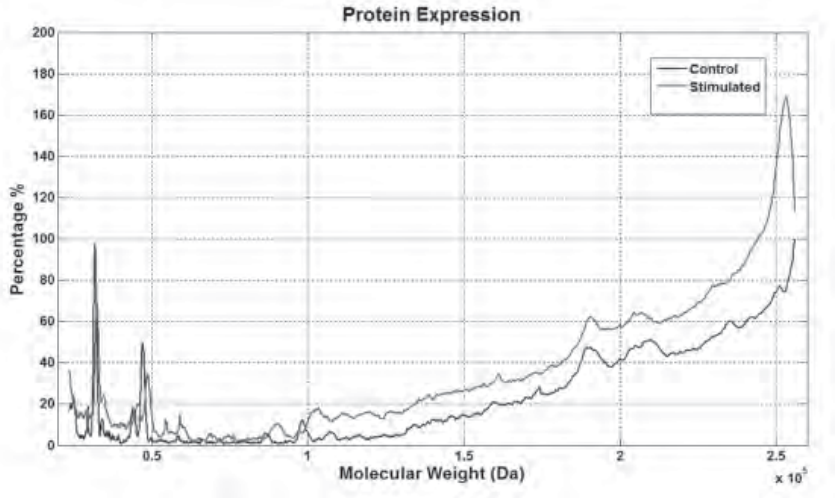

Figure 4. The molecular weight versus the percentage of the protein concentration is shown for the control and stimulated group. The stimulated group showed higher concentrations of proteins in comparison with the control group.

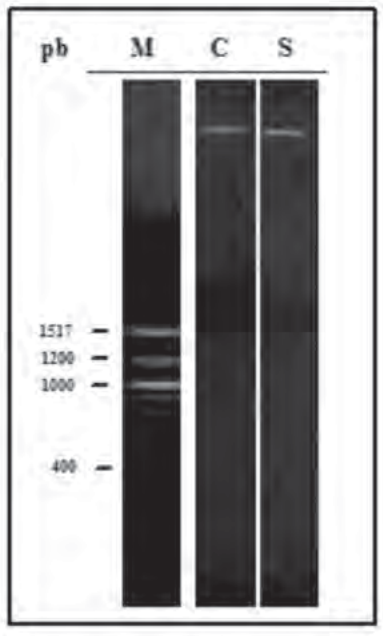

Figure 5. The DNA profile is shown for the groups; control (C), and stimulated (S). (M) represents the molecular markers.

To determine apoptosis, programmed cell death, the fragmentation of the DNA was studied. The genomic DNA profile is shown in Figure 5. The results do not show evidence of DNA fragmentation for the stimulated samples.

DISCUSSION AND CONCLUSIONS

In this paper the interaction between EMF and matter was described as well as the characteristics of magnetic and electromagnetic stimulation. Specifically, this article shows some preliminary data on the stimulation of the neuroblastoma cell line SK- NSH.

These results suggest that MF produce quantitative alterations in protein. The DNA gel results do not show significant variations and there is no evidence of apoptosis when a MF of 19 mT at 8 Hz is used. This result coincide with other studies performed on neuroblastoma where a MF of 1 – 2 mT was applied at the rate of 50 – 60 Hz and no apoptosis was present (Pirozzoli *et al.*, 2003).

In conclusion, this paper shows that a MF of 19 mT at a rate of 8 Hz can produces alterations in the cells. However more research is need to fully conclude the effects of EMF.

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REFERENCES

Beiser A. (2003). *Concepts of Modern Physics*. International Edition, Mc. Graw Hill.

Boal D. (2002). *Mechanics of the cell*. Cambridge.

Casas J.S., Castellano E.E., Ellena J., García-Tasende M.S., Pérez-Parellé M.L., Sánchez A., Sánchez – González A., Sordo J., Toucela A. (2008). New insights on the Lewis acidity of diorganolead (IV) compounds: Diphenyllead (IV) complexes with N, O, S-chelating ligands. Polyhedron. *J of Inorg. Biochem* 102, 33-45.

Challis L.J. (2005). Mechanisms for interaction between RF fields and biological tissue. *Bioelectromagnetics* 7, 98-106.

Dutta S.K., Millis. R.M. (1986). Biological Effects of the Electropollution, Brain Tumors and Experimental Models. *Information Ventures, Philadelphia, PA*.

Funk R. H.W., Monsees T.K. (2006). Effects of Electromagnetic Fields on Cells: Physiological and Therapeutical Approaches and Molecular Mechanisms of Interaction A Review. *Cells Tissues Organs* 182, 59-78.

Glaser R. (1992). Current concepts of the interaction of weak electromagnetic fields with cells. *Bioelectrochemistry and Bioenergetics* 27, 255-268.

Grunder W., Kaiser F., Walleczek J. (1992). Mechanisms of Electromagnetic Interaction with Cellular Systems. *Naturwissenschaften* 12, 551- 9.

Halliday D., Resnick R., Krane K.S. (1999). *FÍSICA* Vol. 2. CECSA.

James A.E., Price R.R., Rollo F.D., Patton J.A., Erickson J.J., Coulam C.M., Partian C.L. (1982). Nuclear Magnetic Resonance Imaging. A Promising Technique. *The journal of the American Medical Association* 247, 1331-1334.

Linde T., Mild K.H. (1997). Measurement of Low Frequency Magnetic Fields from Digital Cellular Telephones. *Bioelectromagnetics* 18, 184 - 186.

Pirozzoli M.C., Marino C., Lovisolo G.A., Laconi C., Mosiello L., and Negoni A. (2003). Effects of 50 Hz Electromagnetic Field Exposure on Apoptosis and Differentiation in a Neuroblastoma Cell Line. *Biolectromagnetics* 24, 510-516.

Tenforde T.S. (1992). Biological Interactions and Potential Health Effects of Extremely-Low Frequency Magnetic Fields from Power Lines and Other Common Sources. *Annual Review of Public Health* 13, 179-196.

Zhadin M.N. (2001). Review of Russian Literature on Biological Action of DC and Low-Frequency AC Magnetic Fields. *Bioelectromagnetics* 22, 27-45.