Ion Cyclotron Bioresonance in Regenerative Medicine

Alberto Foletti[1] **and Settimio Grimaldi**[2]

[1]BITitalia, Milan, Italy
[2]Institute of Neurobiology and Molecular Medicine, CNR, Rome, Italy

Abstract— The prometheus myth, is a fitting model for regenerative medicine. As punishment for giving fire to humanity, Zeus ordered Prometheus chained to a rock and sent an eagle to eat his liver each day. However, Prometheus' liver was able to regenerate itself daily, enabling him to survive. Today we hope to make the legendary concept of regeneration into reality by developing therapies to restore lost, damaged, or aging cells and tissues in the human body. Electromagnetic therapy is a treatment method in which an electromagnetic or magnetic stimulus is used to achieve physiological changes in the body. The specific aim of the present work concerns the effectiveness of low frequency electromagnetic fields treatment (tuned at Calcium cyclotron energy resonance) to modify biochemical properties and trigger cells differentiation in a pituitary cells line (AtT20). Cells were exposed to a 7 Hz electromagnetic field (B_o field 9.2 μT) a commercially available wave generator (Vega Select 719), the cyclotron frequency were calculated by the following equation $f_c = \frac{q}{2\pi m} B_o$, where f_c is the cyclotron frequency, q and m are the charge and mass of the ion, and B_o is the vector of the geomagnetic field (DC field) parallel to the component of the applied electromagnetic field ($B \sin$). In our case since the geomagnetic component (B_o) parallel to the applied $B \sin$ is 9.2 μT, the calculated f_c for calcium is 7 Hz. Here, we report that 50 Hz 2 mT ELF-EMF on rat anterior pituitary derived AtT20 D16V cells produces a sudden increase in the intracellular calcium level, followed by the reorganization of the cytoskeletal network via the polymerization of the actin and the differentiation of the proteins expression. These findings demonstrate that exposure to cyclotron resonance can transfer biological information on pituitary cells, supporting the relevance of low frequency electro-magnetic field as a therapeutic agent, thus suggesting the potential use of cyclotron resonance in nerve regeneratrion.

1. INTRODUCTION

The aim of this work is the study of the effect of electromagnetic radiations (ELF-EMF) at a frequency of 7 Hz on the differentiation process of pituitary corticotrope-derived AtT20 D16V cells. These cells respond to nerve growth factor (NGF) [1] by extending neurite-like processes and differentiating into neurosecretory-like cells. To establish whether exposure to the field could influence the molecular biology of the pituitary gland; a corticotrope-derived cells line (AtT20 D16V) was exposed to ELF-EMF at a frequency of 7 Hz, and a magnetic flux density of 20 micro Tesla (μT).

2. MATERIALS AND METHODS

2.1. Cell Culture

AtT20 D16V cells (American Type Culture Collection, Rockville, MD) were grown in monolayer culture on thin (00) glass cover slips (Corning Glass Works, Corning, NY) coated with poly-L-lysine, or in plastic culture flasks, in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% fetal calf serum, 1.0 unit/ml penicillin, and 1.0 mg/ml streptomycin (Sigma Chemical Company, St. Louis, MO) at 37°C a humidified incubator in 95% air and 5% CO_2. Cells were plated at 250,000/ml.

2.2. ELF-EMF Exposure Systems

Cells were continuously exposed in a small solenoid placed in a cells incubator the field were supplied by a commercial signal generator (Vega select 719).

2.3. Electrophoresis

SDS-polyacrylamide gel electrophoresis (SDS-Page) was carried out according to Laemmli, 1970. After exposure to Calcium ICR electromagnetic fields, equal amount of cell proteins from control and exposed cells, measured by Lowry test, were loaded for each line, after lysis in sample buffer. The samples were boiled for 5 minutes. Electrophoresis was carried on 7.5% SDS polyacrilamide gel at 30 mA for about 2 hours. Gel was subsequently transferred on nitrocellulose membrane (Biorad) at 200 mA for 3 hours, and membrane, after blocking in 3% not dried fat milk for 1 hours at room temperature, was incubated NF-200 antibody (Sigma) at a dilution of 1:100 as suggested by manufacturer and revealed by ECL (Amersham).

Three different sets of experiments were performed.

3. RESULTS

In Fig. 1 is reported the effect of 7 Hz continuous exposure on AtT20cells (right panel) compare with control non exposed cells (left panel). Arrows indicate the formation of neurite like protrusion between cells.

In Fig. 2 is a western blot electrophoresis of the protein extract from 7 Hz cultured and control AtT20 cells. Clearly exposure induces synthesis of the neurite protein NF-200 in the exposed cells, while the NF-200 is almost non present in a control culture.

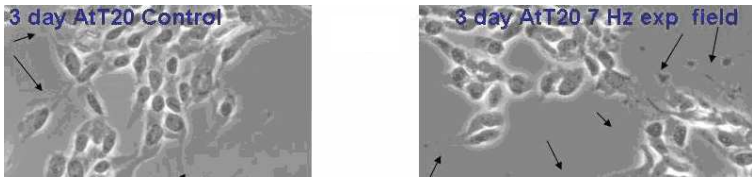

Figure 1: Phase contrast microscopy of 7 Hz exposed AtT20 cells.

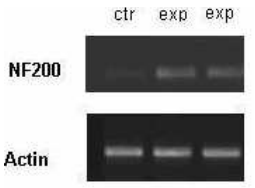

Figure 2: Western blot analysis of control and 7Hz exposed AtT20 cells.

4. CONCLUSIONS

AtT20 D16V is an interesting cell line because it expresses all enzymes required for polysialylation of neural cell adhesion molecule (NCAM). Additionally, the AtT20 D16V clone D16V, is known, to spontaneously develop, after 5 days of culture, long neurite-like processes in the growth cones where ACTH secretory granules accumulate.The findings that in exposed samples, cells more rapidly exhibit properties typical of peptidergic neuronal cells (Fig. 2) is further supported both by the synthesis and accumulation of the neuronal protein NF-200. Our results suggests that exposure to 7 Hz EMF can be used as a tool to initiate differentiation in AtT20 D16V cells.

ACKNOWLEDGMENT

This work has been partially supported by NAMED.

REFERENCES

1. Lisi, A., M. Ledda, E. Rosola, D. Pozzi, E. D'Emilia, L. Giuliani, A. Foletti, A. Modesti, S. J. Morris, and S. Grimaldi, "Extremely low frequency electromagnetic field exposure promotes differentiation of pituitary corticotrope-derived AtT20 D16V cells," *Bioelectromagnetics*, Vol. 27, No. 8, 641–651, 2006.
2. Foletti, A., A. Lisi, M. Ledda, F. De Carlo, and S. Grimaldi, "Cellular ELF signals as a possibile tool in informative medicine," *Electromagn. Biol. Med.*, Vol. 28, No. 1, 71–79, 2009.
3. Liboff, A. R., "Toward an electromagnetic paradigm for biology and medicine," *J. Altern. Complement. Med.*, Vol. 10, No. 1, 41–47, Review, 2004.
4. Lisi, A., S. Rieti, A. Cricenti, A. Flori, R. Generosi, M. Luce, P. Perfetti, A. Foletti, M. Ledda, E. Rosola, L. Giuliani, and S. Grimaldi, "ELF non ionizing radiation changes the distribution of the inner chemical functional groups in human epitelial cell (HaCaT) culture," *Electromagnetic Biology and Medicine*, Vol. 25, 281–289, 2006.
5. Lisi, A., M. Ledda, F. De Carlo, D. Pozzi, E. Messina, R. Gaetani, I. Chimenti, L. Barile, A. Giacomello, E. D'Emilia, L. Giuliani, A. Foletti, A. Patti, A. Vulcano, and S. Grimaldi, "Ion cyclotron resonance as a tool in regenerative medicine," *Electromagnetic Biology and Medicine*, Vol. 27, No. 2, 127–133, 2008.

6. Lisi, A., M. Ledda, F. De Carlo, A. Foletti, L. Giuliani, E. D'Emilia, and S. Grimaldi, "Ion Cyclotron Resonance (ICR) transfers information to living systems: Effects on human epithelial cell differentiation," *Electromagnetic Biology and Medicine*, Vol. 27, No. 3, 230–240, 2008.
7. Lisi, A., A. Foletti, M. Ledda, E. Rosola, L. Giuliani, E. D'Emilia, and S. Grimaldi, "Extremely low frequency 7 Hz 100 μT electromagnetic radiation promotes differentiation in the human epitelial cell line HaCaT," *Electromagnetic Biology and Medicine*, Vol. 25, 269–280, 2006.