Experimental and Numerical investigation of Electromagnetic Field at Different Cancer Cell Lines

Nenad D. Filipovic *Member, IEEE*, Tijana R. Djukic, Milos Radovic, Danijela Cvetkovic, Snezana Markovic and Branislav Jeremic

Abstract— **There is a strong interest of investigation of Extremely Low Frequency (ELF) ElectroMagnetic (EM) fields in the clinic. In this study we investigated experimentally in-vitro and in-sillico with computer simulation influence of 50 Hz EM field at three different cancer cell lines: breast cancer MDA-MB-231 and colon cancer SW-480 and HCT-116.**
Computer reaction-diffusion model with the net rate of cell proliferation and effect of electromagnetic field in time was developed. The fitting procedure for estimation of the computer model parameters was implemented.

Experimental and computer model data have shown good comparison. These findings can open a new avenue for better controlling the growth of cancer cells at specific frequencies without affecting normal tissues, which may have a great influence in clinical oncology.

I. Introduction

Alternating electric fields have shown a wide range of effects on living tissues. Depending on frequencies, they have been able to stimulate excitable tissues (on very low frequencies under 1 KHz) being used for nerve, muscle or heart stimulation [1,2], or, as is the case with low-frequency pulsed electric fields, to stimulate bone growth and accelerate fracture healing [3]. On very high frequencies (>>MHz), they have been used for diathermy and radiofrequency tumor ablation [4]. Intermediate-frequency electric fields (>10KHz to MHz) were mostly considered as having no over biological effect [5] and, hence, medical application, though several non-thermal cellular effects have been observed [6-8].

Last decade also brought a number of *in-vitro* and *in-vivo* studies which concentrated on the antiproliferative/ anticancer effects of alternating electric fields [9-11]. They clearly documented that arrest of cell proliferation and cell destruction is observed when low-intensity, intermediate frequency (100-300 KHz) alternating electric fields is applied as well as when amplitude-modulated electromagnetic fields of somewhat lower frequencies (0.1 Hz to 114 KHz) [12]. These interesting results led to implementation of such approaches in humans, and initial results on a number of tumor entities/sites (recurrent glioblastoma multiforme, hepatocellular carcinomas, breast carcinomas, other) were encouraging [10,12,13]. Another important aspect in cancer cell death, apoptosis, can be considered as a regulated process. Hence, there are a lot of investigations in the regulation of apoptosis [14,15]. In one of these, Zhang et al 2002 [16] showed that an ELF Pulsed-gradient magnetic field can induce apoptosis in cancer cells, and that it may also block the development of neovascularization required for tumor supply.

All computer models have the common aim of predicting certain features of tumor growth in the hope of finding new ways to either control, stop or reverse neoplastic progression. The golden aim of computer modeling is to make a model which yields reproducible and accurate predictions, the effects of different genetic, epigenetic and environmental changes, as well as the impact of therapeutically targeting different aspects of the tumor.

Treatment plans based on the use of such computer modeling and simulation processes will require rigorous validation studies, regulatory approvals and, then, eventual integration into the clinical practice [17].

In this study we investigated electromagnetic fields at specific frequencies for three different cell lines: breast cancer MDA-MB-231 and colon cancer SW-480 and HCT-116. The paper is organized as following. Firstly in the method section experimental setup was described. Then, numerical methods for simulation and optimization of the electromagnetic field influence on the cancer cell are given. Results of experiments and computer simulation data are compared. Finally some conclusions are described.

II. Experimental and Numerical Methods

A. Drugs

Human breast cancer cell line MDA-MB-231 and colon cancer cell lines SW-480 and HCT-116 were exposed to 50 Hz EMF using *in vitro* exposure systems for 24 and 72 h.

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N. D. Filipovic is with the Faculty of Engineering, University of Kragujevac, Serbia (phone: +381-34-334379; fax: +381-34-333192; e-mail: fica@ kg.ac.rs).

T. R. Djukic and M. Radovic, are with the Faculty of Engineering, University of Kragujevac and BioIRC Kragujevac, Serbia (e-mails: tijana@kg.ac.rs, mradovic@kg.ac.rs).

D. Cvetkovic and S. Markovic, are with the Faculty of Science, University of Kragujevac, Serbia (e-mails: c_danijela@yahoo.com, smarkovic@kg.ac.rs).

B. Jeremic is with Stellenbosch University and Tygerberg Hospital, Cape Town, South Africa, (e-mail: bjeremic@sun.ac.za).

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B. Cell preparation and culturing (UM.01, UM.02, UM.03, UM.04)

Breast cancer cell line MDA-MB-231 and colon cancer cell lines SW-480 and HCT-116 were obtained from the American Tissue Culture Collection (Manassas, VA, USA). These cells were propagated and maintained in DMEM (Dulbecco's Modified Eagle Medium), (Gibco, USA) and supplemented with 10% fetal bovine serum (PAA), antibiotics 100 IU/mL penicillin and 100 µg/mL streptomycin. Cells were growth in 75 cm^2 culture bottles supplied with 15 ml DMEM, and after a few passages cells were seeded in 96-well plate. Cells were cultured in a humidified atmosphere of 5% CO_2 at 37 °C. All studies were done with cells at 70 to 80% confluence [18,19].

C. Statistical analysis

The data are expressed as the means ± standard errors (SE). Biological activity is result of one individual experiment, performed in triplicate. The magnitude of correlation between variables was done using a SPSS (Chicago, IL) statistical software package (SPSS for Windows, ver. 17, 2008).

D. Numerical modeling of effect of electromagnetic field on cancer cells

The basic goal of this study was to develop a mathematical model that will be capable to accurately predict the behavior of cancer cell both with and without the electromagnetic field acting on cell lines. For this purpose the model proposed by Swanson et al 2003 [20] was used. This fundamental model is mainly used for brain tumors, but it can be used to model the behavior of any tumor population in general [21]. However, the Swanson's model is designed to describe the untreated tumor growth. Extensions of this model were proposed to include the effects of radiation therapy [21], resection [22] and chemotherapy [23]. In this paper, the fundamental model was extended to take into account the effect of electromagnetic field.

The basic equation of the extended model is given by:

$$\frac{\partial \mathbf{c}}{\partial t} = D\frac{\partial^2 \mathbf{c}}{\partial \mathbf{x}^2} + \rho \mathbf{c} - F(t)\mathbf{c} \qquad (1)$$

where $c = c(\mathbf{x}, t)$ is the concentration of tumor cells, D is the spatially constant diffusion coefficient, ρ is the net rate of cell proliferation and $F(t)$ represents the effect of electromagnetic field at time t.

The initial and boundary conditions are set such that at $t = 0$ the cell concentration is set to c_0 and the zero flux is prescribed on the boundary of the observed domain - $\mathbf{n} \cdot \nabla c = 0$. In-house developed software was used for imaging analysis of experimental results (images obtained using the procedure described in Section Experimental setup). All the areas of cells visible on experimental images are summed and the cumulative single cell that has the same area is created. The initial cell concentration is determined using this software and images from the beginning of the experiment. The same procedure was used to evaluate the percentage of cells over the whole observed domain and this percentage was later used during fitting procedure explained in Section Experimental setup.

Since the effect of electromagnetic field is represented by an unknown function, it was necessary to estimate the function itself and the parameters. Again using the experimental results, the logaritmic function of the following form was utilized to model the effect of electromagnetic field:

$$F(t) = a\ln(t) + b \qquad (2)$$

where parameters a and b have to be estimated.

Parameters describing the diffusion and proliferation of cancer cells are different for different types of tumor and should be estimated accordingly. Hence, there are four parameters that need to be estimated using the experimental results - D, ρ, a and b.

The equation (1) was solved numerically using the finite element method [24]. The in-house developed software for time-dependent two-dimensional analysis was used for this purpose. The incremental-iterative form of equation (1) for time step Δt and equilibrium iteration "i" is given by:

$$\left[\frac{1}{\Delta t}\mathbf{M}_c + {}^{t+\Delta t}\mathbf{K}_{cc}^{(i-1)}\right] \cdot \Delta \mathbf{c}^{(i)} = {}^{t+\Delta t}\mathbf{F}_c^{(i-1)} \qquad (3)$$

The left upper index "$t + \Delta t$" denotes that the quantities in question are calculated at the end of a time step. The matrix $\mathbf{M}_c$ is the mass matrix, $\mathbf{K}_{cc}$ is the diffusion matrix (here are also included the effects of electromagnetic field) and $\mathbf{F}_c$ is the forcing vector, that takes into account the boundary conditions.

III. Results

Our study showed that used electromagnetic field has a strong antiproliferative effect on treated cancer cell lines. 50 Hz EMF has given a statistically significant reduction in the

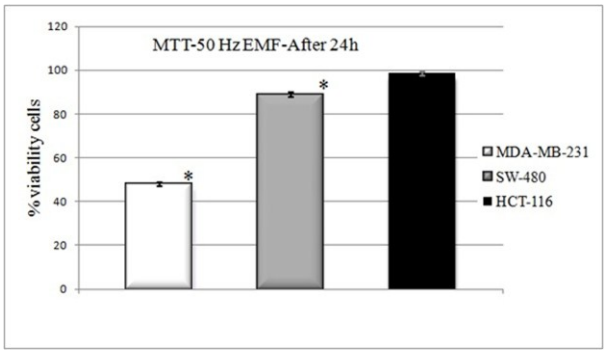

Fig. 1. The histogram of effects of 50 Hz EMF on human breast cancer cell line MDA-MB-231 and colon cancer cell lines HCT-116 and SW-480. The antiproliferative effect was measured by MTT assay after 24 h of exposure. All values are mean ± SEM, n=3, *p < 0.05 as compared with with control (100%)

number of cancer cells after 24 h (Fig. 1) and 72 h of treatment (Fig. 2). Most pronounced antiproliferative effect

was obtained in the breast cancer cell line MDA-MB-231 where the percentage of cell viability after 24 h was 47.73%, and after 72 h it was 80.28%. Significantly weaker effect was observed in colon cancer cell lines, so percent of cell viability in cell line SW-480 after 24 h was 88.53% and after 72 h it was 94.19%, whereas percent of cell viability in cell line HCT-116 after 24 h was 98.28% and after 72 h it was 97.20%.

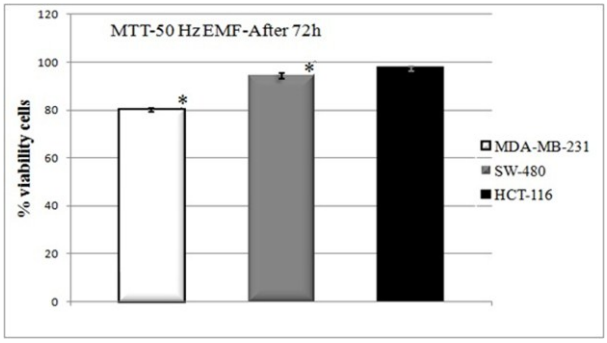

Fig. 2. The histogram of effects of 50 Hz EMF on human breast cancer cell line MDA-MB-231 and colon cancer cell lines SW-480 and HCT-116. The antiproliferative effect was measured by MTT assay after 72 h of exposure. All values are mean ± SEM, n=3, *$p <$ 0.05 as compared with control (100%).

Fig. 3 shows the diagram of variation of cell percentage over the whole domain with respect to time. The red line represents the control cancer cell line, where no electromagnetic field was applied. The blue line represents the cancer cell line with electromagnetic field acting on cells. The black line represents the effect of electromagnetic field, that was modeled using logarithmic function described in equation (2). The behavior of cells is modeled using finite element as described. The results obtained using the mentioned approaches are shown for three different states in time above the diagram in Fig. 3. These specific moments in time are chosen because during experiments state of cells was pictured in these moments and those images are also shown in Fig. 3.

IV. Discussion and Conclusion

Aim of this study was to investigate influence of low level frequency of electromagnetic field on three different cancer cell lines: breast cancer MDA-MB-231 and colon cancer SW-480 and HCT-116. The analysis was performed experimentally and by computer simulation.

After 24 h of incubation cells were exposed to 50 Hz radiofrequency electromagnetic field using *in vitro* exposure systems for 24 and 72 h. We developed a specific reaction-diffusion model with the net rate of cell proliferation and effect of electromagnetic field in time. Also the fitting procedure for estimation of the computer model parameters was applied.

From experimental results it was clearly showed disintegration of cells which were treated by electromagnetic field of 50 Hz frequency compared with untreated control cells. These results add to the existing body of knowledge in this field. Following encouraging own *in vitro* and *in vivo* results [9], Kirson et al (2007) [10] embarked on seemingly first-ever application of alternating electric fields in 10 patients with recurrent glioblastoma multiforme. The median time to disease progression was 26.1 weeks (range, 3-124 weeks) and the progression-free survival time at 6 months was 50%. The median overall survival time was 62.2 weeks at the time of the report (range, 20.3-124 weeks). This treatment approach offered an improvement which was more

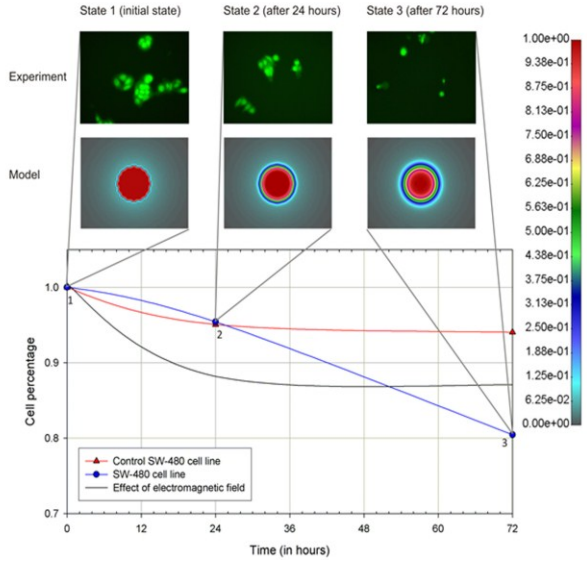

Fig. 3. The experimental images, obtained numerical results and diagram of cell percentage variation with respect to time

than double when compared to historic controls. No serious adverse events (SAE) were observed, while 9 out of 10 patients experienced mild to moderate (grade 1 and 2, respectively) contact dermatitis beneath the electrode gel, all successfully treated with topical steroid creams and periodic electrode relocation.

In an interesting approach, Barbault et al [12] tried to identify tumor-specific frequencies tested the feasibility of administering such frequencies to patients with advanced cancer using a noninvasive biofeedback method to identify such tumor-specific frequencies. Of a total of 163 examined patients with the diagnosis of cancer, a total of 1524 frequencies ranging from 0.1 Hz to 114 KHz were identified. Most frequencies (57-92%) were specific for a single tumor type. Treatment was offered to 28 patients (26 treated in Switzerland and 2 in Brazil), who administered treatment three times a day by themselves. Thirteen patients were evaluable for response. Of two patients with hormone-refractory metastatic breast cancer one had a complete response lasting 11 months, while second one had a partial response lasting for 13.5 months. Four patients had stable disease which lasted from 4.0 to >34.1 months, while only one patient experienced grade 1 fatigue during or immediately after the treatment(s).

The largest study so far has recently been published by Costa et al (2011) [13] which included 41 patients with advanced hepatocellular carcinoma treated with low levels of electromagnetic fields modulated at specific frequencies (27.12 MHz). Three-daily outpatient treatments were

administered until disease progression or death. The majority of these patients had either failed standard treatment options or had severely impaired liver function that limited their ability to tolerate any form of systemic or intrahepatic therapy. Fourteen (34.1%) patients had stable disease for > 6 months. Median progression-free survival was 4.4 months and median overall survival time was 6.7 months. There were no grade 2-5 treatment-related toxicities observed during this study. This data seems comparable to recent data from various chemotherapy and/or targeted therapy in this setting [13].

Our experimental results from florescence microscopic images clearly showed nuclear disintegration of cells which were treated by electromagnetic field of 50 Hz frequency compared with untreated control cells. It was shown that a large percentage of treated cells results in increased early apoptosis after 24 h and 72 h, compared to the control group cells.

We have developed computer model that is intended to simulate influence of electromagnetic field on the cancer cells. The images of experimental setup were used to create the cumulative single cells for computer simulation. Of course it is our first approximation and we got very good results in comparison with the *in vitro* experiments on the different cancer cell lines. However, any computer model that can be used in the clinic must go into precise predicting tumor size and shape. Also future computer models have to predict the changes induced in the host by the growing tumor and the impact that multiple treatment strategies would have on halting the progression of the tumor growth.

These exciting findings of the influence of electromagnetic field on the inhibition of proliferation of cancer cells may give the potential application of the appropriate frequencies of electromagnetic field in oncology. Computer simulation tools also uncover a new avenue to optimize and control tumor growth and may have broad implications for the treatment of cancer. These results call for additional in vivo investigations before being tested in a phase I-II clinical trial.

REFERENCES

[1] C. Polk, "Therapeutic applications of low-frequency sinusoidal and pulsed electric and magnetic fields," In: Bronzino JD (ed). *The biomedical engineering handbook*. Boca Raton, FL: CRC Press, 1995,pp. 1404-1406.

[2] Y. Palti, "Stimulation of internal organs by means of externally applied electrodes," *J Appl Physiol* 1966, 21, pp. 1619-1623.

[3] C.A. Besset, "The development and application of pulsed electromagnetic fields (PEMFs) for ununited fractures and arthrodesis," *Clin Plast Surg* 1985, 12, pp. 259-277.

[4] C.K. Chou, "Radiofrequency hyperthermia in cancer therapy," In: Bronzino JD (ed). *The biomedical engineering handbook*. Boca Raton, FL: CRC Press, 1995, pp. 1424-1430.

[5] E. Elson, "Biologic effects of radiofrequency and microwave fields in vivo and in vitro experimental results," Bronzino JD (ed). *The biomedical engineering handbook.* Boca Raton, FL: CRC Press, 1995, pp. 1417-1423.

[6] U. Zimmerman, J. Vienken, G. Piwat, "Rotation of cells in an alteranating electric field: the occurrenc eof a resonance frequency,". *Z naturforsch C* 1981, 36, pp. 173-177.

[7] C. Holzapfel, J. Vienken, U. Zimmermann, „Rotation of cells in an alternating electric field: theory and experimental proof," *J Membr Biol* 1982,67, pp. 13-26.

[8] P. Pawlowski, I. Szutowicz, P. Marszalek, M. Fikus. "Bioelectrorheological model of the cell," 5. Electrodestruction of the cellular membrane in alternating electrical field. *Biophys J* 1993,65, pp. 541-549.

[9] E.D. Kirson, Z. Gurvich, R. Schneiderman, E. Dekel, A. Itzakhi, Y. Wasserman, R. Schatzberger, Y. Palti, "Disruption of cancer cell replication by alternating electric fields," *Cancer Res* 2004,64, pp. 3288-3295.

[10] E.D. Kirson, V. Dbaly, F. Tovarys, J. Vymazal, J.F. Soustiel, A. Itzakhi, D. Mordechovich, S. Steinberg-Shapira, Z. Gurvich, R. Schneiderman, Y. Wasserman, M. Salzberg, B. Ryffel, D. Goldsher E. Dekel, Y. Palti, "Alternating electric fields arrest cell proliferation in animal tumor models and human brain tumors," *Proc Natl Acad sci* 2007,104, pp. 10152-10157.

[11] J.W. Zimmerman, M.J. Pennison, I. Brezovich, N. Yi, C.T. Yang, R. Ramaker, D. Absher, R.M. Myers, N. Kuster, F.P. Costa, A. Barbault, B. Pasche, cancer cell proliferation is inhibited by specific modulation frequencies. *Br J Cancer* 2012,106, pp. 307-313.

[12] A. Barbault, F.P. Costa, B. Bottger, R.F. Munden, F. Bomholt, N. Kuster, B. Pasche, "Amplitude-modulated electromagnetic fields for the treatment of cancer: discovery of tumor-specific frequencies and assessment of a novel therapeutic approach," *J Exper Clin Cancer Res* 2009, pp. 28-51.

[13] F.P. Costa, A.C. de Oliveira, R. Meirelles, M.C.C. Machado, T. Zanesco, R. Surjan, M.C. Chammas, M. de Souza Rocha, D. Morgan, A. Cantor, J. Zimmerman, I. Brezovich, N. Kuster, A. Barbault, B. Pasche," Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. *Brt J Cancer* 2011,105, pp. 640-648.

[14] M. Fang, H.Q. Zhang, S.B. Xue, "Role of Calcium in apoptosis of HL-60 cells induced by harring tonine," *Science in China, Ser. C* 1998,41, pp. 600–607.

[15] C.P. Silva, C.R. Oliveira, M.C.P. Lima, "Apoptosis as a mechanism of cell death induced by different chemotherapeutic drugs in human leukemic T-lymphocytes," *Biochem Pharmacol* 1996,51, pp. 1331–1340.

[16] X. Zhang, H. Zhang, C. Zheng, C. LI, X. Zhang, W. Xiong, Extremely Low Frequency (ELF) Pulsed-Gradient Magnetic Fields Inhibit Malignant Tumour Growth at Different Biological Levels, *Cell Biology International* 2002,26(7), pp. 599–603.

[17] J.L. Gevertz, G.T. Gillies and S. Torquato, "Simulating tumor growth in confined heterogeneous environments," *Phys. Biol.* 2008, 036010 (10pp) doi:10.1088/1478-3975/5/3/036010

[18] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *J Immunol Meth* 1983,65, pp. 55-63.

[19] D. Baskić, S. Popović, P. Ristić, N.N. Arsenijević, "Analysis of cycloheximide-induced apoptosis in human leukocytes: Fluorescence microscopy using annexin V/propidium iodide versus acridin orange/ethidium bromide," *Cell Biol Int* 2006,30(11), pp. 924-932.

[20] K.R. Swanson, C. Brigde, J.D. Murray, A.C. Jr. Ellsworth, "Virtual and real brain tumors: using mathematical modeling to quantify glioma growth and invasion," *J. Neurol. Sci.* 2003,216, pp. 1-10.

[21] R. Rockne, E.C.Jr. Alvord, J.K. Rockhill, K.R. Swanson, "A mathematical model for brain tumor response to radiation therapy," *J. Math. Biol*. 2009,58(4-5), pp. 561-578.

[22] D.E. Woodward, J. Cook, P. Tracqui, G.C. Cruywagen, J.D. Murray, E.C. Jr. Alvord,. "A mathematical model of glioma growth: the effect of extent of surgical resection," *Cell Prolif*. 1996,29, pp. 269-288.

[23] P. Tracqui, G.C. Cruywagen, D.E. Woodward, G.T. Bartoo, J.D. Murray, E.C.Jr. Alvord, "A mathematical model of glioma growth: the effect of chemotherapy on spatio-temporal growth,".*Cell Prolif* 1995,28, pp. 17-31.

[24] N. Filipovic, A. Peulic, N. Zdravkovic, V. Grbovic-Markovic and A. Jurisic-Skevin, "Transient Finite Element Modeling of Functional Electrical Stimulation," *General Physiology and Biophysics*, 2011,30(1), pp. 59-65.