INFLUENCE OF 864 MHZ ELECTROMAGNETIC FIELD ON GROWTH KINETICS OF ESTABLISHED CELL LINE

Ivan Pavicic, Ivancica Trosic,

Institute for Medical Research and Occupational Health, Ksaverska cesta 2, HR-10001 Zagreb, CROATIA, ipavicic@imi.hr

Abstract: *Considering often contradictory data on biological effects of mobile phones frequencies on established cell culture lines, our study aimed at evaluating the influence of 864 MHz electromagnetic field on proliferation, colony forming ability and viability of Chinese hamster lung cells of line V79 cell. Prior to exposure for 1, 2 and 3 hours in transversal electromagnetic mode cell (TEM-cell) cell culture were sub-cultivated for one day. Cells were exposed to 864 MHz continuous wave (CW) at an average specific absorption rate (SAR) of 0.66 W/kg. Philips PM 5508 connected with signal amplifier was used as signal generator. To determine cell growth, V79 cells were plated in concentration of $1x10^4$cells/ml, and raised in a humified atmosphere at 37 °C in 5% CO_2. Cell proliferation was determined by cell counts for each hour of exposure on post-exposure days 1, 2, 3, 4 and 5. To identify colony-forming ability, cells were cultivated in concentration of 40 cells/ml, and incubated as above. Colony forming ability for each hour of exposure was defined by colony counts on experimental day 7. Trypan blue exclusion method was used to determine cell viability. In comparison to sham-exposed cells, growth curve of irradiated culture samples showed significant decrease ($p<0.05$) after 2 and 3 hours of exposure on experimental day 3, respectively. Both, the colony forming ability and viability of irradiated cells did not significantly differ from sham-exposed controls. In conclusion, no vigorous influence of 864 MHz electromagnetic field on growth kinetics of V79 cell culture was detected. Where effect was seen, it was not dose-dependent.*

Key words: *864 MHz microwave, TEM cell, V79 cell culture, growth kinetics*

1. INTRODUCTION

Common concern on biological effects of microwaves grows because of increased general use of microwave generating systems. Many reported biological effects of radiofrequency/microwave (RF/MW) in humans are simply indeterminate with respect to their significance to health [1]. Therefore, it is important that biological effects be understood, at least to the stage of their clinical significance, so that health-hazard potential can be assessed. Because the potential impact of RF/MW fields on human health is not yet well characterized, the basic cognitions derived from laboratory studies based on cellular and animal test systems is of invaluable significance. Numerous *in vitro* biological effects at non-thermal levels of RF/MW fields have been reported, including influence on cell proliferation, morphology, gene expression, chromosome damage and apoptosis [2,3,4,5,6,7]. Results from

different studies are still inconclusive and conflicting. Considering often contradictory data on biological effects of mobile phones frequencies on established cell culture lines, our study aimed at evaluating the influence of 864 MHz electromagnetic field on proliferation, colony forming ability and viability of V79 cells, lung fibroblasts of Chinese hamster.

2. MATERIALS AND METHODS

V79 cell line, lung fibroblasts of Chinese hamster was used. The cells were routinely cultured in culture medium (RPMI 1640 medium supplemented with antibiotics and fetal calf serum, SIGMA Chemical CO, St. Louis, USA). The cells were maintained in a humified atmosphere at 37°C in 5% CO_2, and pre-incubated for 24 hour prior to the start of the assay. Optimized exposure device, transversal electromagnetic mode cell (TEM-cell) supported with Philips PM 5508 signal generator and signal amplifier was used as RF/MW transmitter [8]. TEM-cell was designed to emits signal frequency of 864 MHz continues waves. The electric field strength inside TEM-cell was 7.3 V/m, and average field power density was 0.1 W/m^2. During the experiment, temperature was measured in 10 minute intervals in order to manage steady condition of 37 ^{0}C inside the exposure device. The values of dielectric parameters of the individual substance of a typical mammalian cell were averaged in accordance with their volume fraction in the live cell, in order to obtain SAR for a single cell [9]. The average SAR was calculated to be 0.66 W/kg [10]. Cell culture has been prepared to be exposed to radiofrequency field of 864 MHz for 1, 2 and 3 hours. To determine cell proliferation V79 cells were plated in concentration of $1x10^4$cells/ml. Cell proliferation was determined microscopically by cell counts for each hour of exposure on post-exposure days 1, 2, 3, 4 and 5. To determine colony-forming ability, cells were plated in concentration of 40 cells/ml. Cell cultures were irradiated for 1, 2 and 3 hours, and cultivated for a period of 7 days. Cell colonies were stained with Giemsa dye, and determined microscopically (10x magn). Trypan blue exclusion method (SIGMA Chemical CO, St. Louis, USA) was used to determine cell viability. Non-viable and viable cell rate were determined by counting cells after microwave radiation on five consecutive days [11]. Analysis of variance (ANOVA/MANOVA) was used for statistical data evaluation.

3. RESULTS AND DISCUSSION

Growth curves of sham-exposed controls and V79 cell culture after one, two and three hours of 864 MHz microwave irradiation are present at Figures 1, 2 and 3. Significant difference was found in cultures that have been exposed for two and three hours in comparison to sham-exposed control cultures (Fig. 2 and 3). Significant decrease in cell number has been noticed on third day following microwave exposure ($p<0.05$). The cell doubling time did not differ between the groups throughout the experiment. Colony forming ability of sham-exposed controls and V79 cells exposed to 864 MHz frequency for 1, 2 and 3 hours has been shown at Figure 4. Colony-forming ability in between sham-exposed and irradiated cell culture samples did not resulted in significant differences, neither significant

difference was found between applied doses or tested exposure times. Figure 5 presents a cell viability of sham-exposed controls and V79 cell culture after 1, 2 and 3 hours of 864 MHz microwave exposure. Applied microwaves did not influence cell viability, since this parameter ranged from 98.5% to 100%, both, in exposed and control cells. Each data point in the curve represents the mean value obtained by six separate samples, both, microwave and sham-exposed cell cultures.

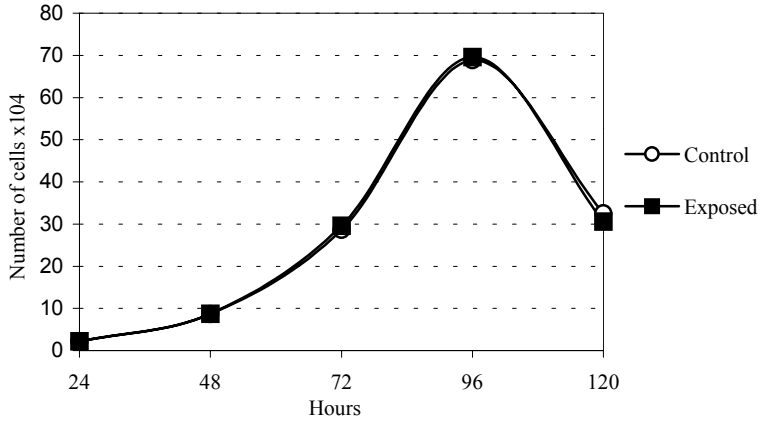

Fig 1. Growth curve of sham-exposed controls and V79 cell culture after one-hour of 864 MHz microwave exposure

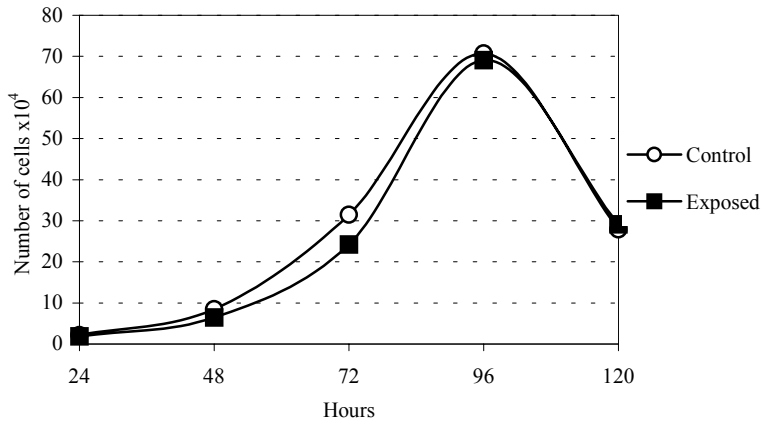

Fig 2. Growth curve of sham-exposed controls and V79 cell culture after two-hour of 864 MHz microwave exposure

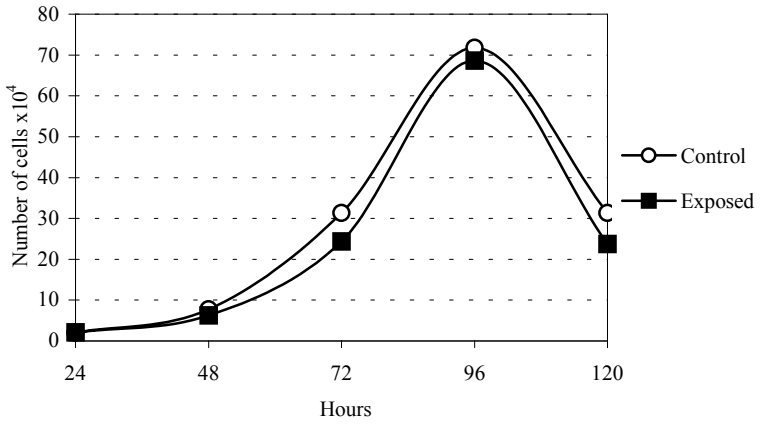

Fig 3. Growth curve of sham-exposed controls and V79 cell culture after three-hour of 864 MHz microwave exposure

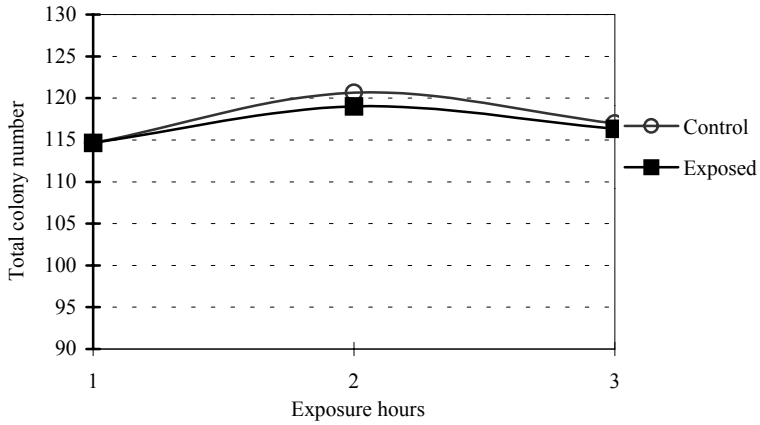

Fig 4. Colony forming ability of sham-exposed controls and V79 cell culture after 1, 2 and 3 hours of 864 MHz microwave exposure

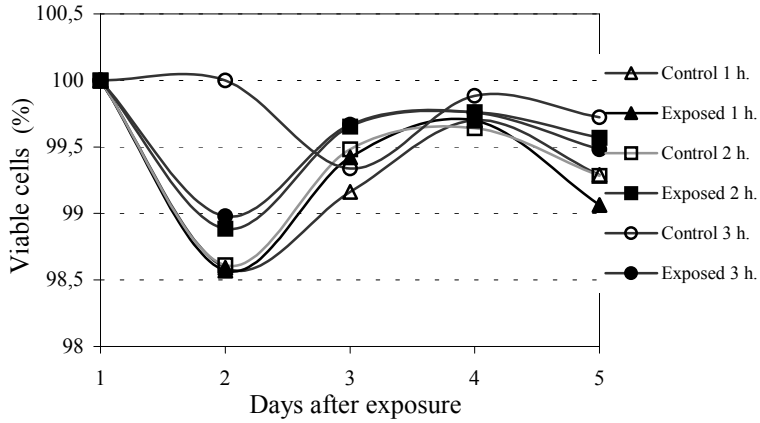

Fig 5. Cell viability of sham-exposed controls and V79 cell culture after 1, 2 and 3 hours of 864 MHz microwave exposure

Selected RF/MW radiation and controlled conditions applied in this study has affected cell proliferation without changing colony forming ability and viability of cell in established culture line. Obtained results are similar to findings referred by Kwee and Rasmark who found growth suppression of human epithelial amnion cells exposed to 960 MHz and SAR value among 0.021 and 2.1 W/kg. The same authors found that there existed so-called "window" effect, i.e., the maximum effect on changes in proliferation rate were at a specific electromagnetic field and exposure time [12]. Velizarov et al. also reported a significant change in cell proliferation in the RF/MW exposed cells in comparison to the non-exposed cells at 39°C and 35°C. Altered cell proliferation was attributed to electromagnetic field exposure but not to the influence of temperature. [13]. French et al was investigating effect of 835 MHz on human astrocytoma cell line at power density of 40 and 8.1 $mWcm^{-2}$. The alteration in cell proliferation was observed at 8.1, whereas alteration in cell morphology was noticed at field power density of 40 mW/cm^{-2}[14]. Otherwise, Stagg et al, and Higashikubo et al. did not observe changes in the transit of cells through G_1, G_2 and S phase, including cell division, immediately after the cells were exposed to frequency modulated continuous wave at 835.62 MHz, at an average SAR of 0.6 W/kg, for up to 100 h [15, 16]. Nevertheless, the capability of low power RF/MW radiation to induce reversible alteration in cell protein structure has been proven [17]. The consequence of changes in kinetics of protein folding and unfolding caused by external irradiation could trigger compensatory or adaptive cell response. Transient decrease in growth kinetics without disturbing viability and colony forming ability of cells pointed out that influence of applied RF/MW of 864 MHz frequency radiation on fundamental biological processes is almost certain, and similar to any biological stressor.

4. CONCLUSION

Obtained findings did not reveal robust effect of 864 MHz electromagnetic field on growth kinetics of V79 cell culture. Where effect was seen, it was not dose-dependent.

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REFERENCES

[1] JM Elwood, Epidemiological studies of radio frequency exposures and human cancer, *Bioelectromagnetics,* S6, 2003, 63-73
[2] RB Stagg, WJ Thomas, RA Jones, WR Adey, DNA Synthesis and Cell Proliferation in C6 Glioma and Primary Glial Cells Exposed to a 836.55 MHz Modulated Radiofrequency Field, *Bioelectromagnetics,* 18, 1997, 230–236.
[3] S Pacini, M Ruggiero, S Iacopo, S Aterini, F Gulisano, M Gulisano, Exposure to global system for mobile communication (GSM) cellular phone radiofrequency alters gene

expression, proliferation, and morphology of human skin fibroblasts, *Oncology Researc/Anti-cancer drug design,* 13, 2002, 19-24
[4] M Port, M Abend, B. Romer, D Van Beuningen, Influence of high-frequency electromagnetic fields on different modes of cell death and gene expression, *International Journal of radiation biology*, 79 (9), 2003, 701-708
[5] PC Goswami, Albee LD, AJ Parsian, JD Baty, EG Moros, WF Pickard, JL Roti Roti, CR Hunt, Proto-oncogene mRNA levels and activities of multiple transcription factors in C3H 10 T ½ murine embryonic fibroblasts exposed to 835.62 and 847.74 MHz cellular phone communication frequency radiation, *Radiation Research* 151(3), 1999, 300-309
[6] O Zeni, AS Chiavoni, A Sannino, A Antolini, D Forgio, F Bersani, Lack of genotoxic effects (micronucleus induction) in human lymphocytes exposed *In vitro* to 900 MHz electromagnetic fields. *Radiation research*, 160, 2003, 152-158
[7] A Peinnequin, A Piriou, J Matheiu, V Dabouis, C Sdebbah, R Malabiau, JC Debouzy, Non-thermal effects of continuous 2.45 GHz microwave on Fas-induced apoptosis in human Jurkat T-cell line, *Bioelectrochemistry*, 51, 2000, 157-161
[8] K Malaric, J Bartolic, Design of a TEM-cell with Increased Usable Test Area, *Turkish Journal of electrical Engineering & Computing Sciences*, 11 (2003), 2; 143-154
[9] KV Steffensen, P Raskmark, GF Penersen, FDTD calculations of the EM-field distribution in a microtiter suspension well, *COST 244: Biomedical Effects of Electromagnetic fields,* Kuopio, 1995, 80-87
[10] World health organization, *Electromagnetic Fields (300 Hz to 300 GHz), Environmental Health Criteria 137,* World Health Organization, Geneva, 1993
[11] RI Freshney, Culture of animal cells, A manual of basic technique. 2000
[12] S Kwee, P Rasmark, Changes in cell proliferation due to environmental non-ionizing radiation 2. Microwave radiation, *Bioelectrochemistry and Bioenergetics*, 44, 1998, 251-255.
[13] S Velizarov, P Rasmark, S Kwee, The effects of radiofrequency fields on cell proliferation are non-thermal, *Bioelectrochemistry and Bioenergetics*, 48, 1999, 171-180
[14] PW French, M Donnellan, DR McKenzie, Electromagnetic radiation at 835 MHz changes morphology and inhibits proliferation of a human astrocytoma cell line, *Bioelectrochemistry and bioenergetics, 4*3, 1997, 13-18
[15] Higashikubo, M Ragouzis, EG Moros, WL Straube, JL Roti Roti, Radiofrequency electromagnetic fields do not alter the cell cycle progression of C3H $10T^{1}/_{2}$ and U87MG cells, *Radiation Research*, 156, 786-795
[16] RB Stagg, WJ Thomas, RA Jones, WR Adey, DNA synthesis and cell proliferation in C_6 glioma and primary glial cells exposed to a 836.55 MHz modulated radiofrequency field, *Bioelectromagnetics*, 18, 1997, 230-236
[17] H Bohr, J Bohr, Microwave enhanced kinetics observed in ORD studies of a protein, *Bioelectromagnetics*, 21, 2000, 68-72R