Original Scientific Paper

COMPARISON OF 864 MHz AND 935 MHz MICROWAVE RADIATION EFFECTS ON CELL CULTURE*

Ivan PAVIČIĆ[1], Ivančica TROŠIĆ[1], and Antonio ŠAROLIĆ[2]

Institute for medical research and occupational health[1], Faculty of Electrical Engineering and Computing, University of Zagreb[2], Zagreb, Croatia

Received in May 2005
Accepted in February 2006

The objective of this study was to compare the effects of 864 MHz and 935 MHz radiofrequency/microwave radiation on the ability of V79 cells to proliferate, form colonies and on their viability. For one, two and three hours, the cells were exposed to the 864 MHz field in a transversal electromagnetic mode cell (TEM) connected with amplifier and to the 935 MHz field in a gigahertz transversal electromagnetic mode cell (GTEM) equipped with a signal generator. The average specific absorption rate (SAR) was 0.08 W kg^{-1} for the 864 MHz field and 0.12 W kg^{-1} for the 935 MHz field. In comparison to the control cell samples, the growth curve of the 864 MHz irradiated cells showed a significant decrease after two-hour and three-hour exposure on the Day 3 after exposure. Likewise, cells exposed to 935 MHz microwaves for three hours showed a significant growth on Day 3 after exposure. The colony-forming ability and viability of cells exposed to 864 MHz and 935 MHz microwaves did not significantly differ from the matched controls. The applied RF/MW fields showed a similar effect on cell culture growth, colony-forming ability and viability of V79 cells.

KEY WORDS: *cell growth, colony-forming ability, GTEM-cell, microwave exposure, TEM-cell, V79 cells, viability*

Sources of radiofrequency/microwave (RF/MW) radiation, particularly mobile phones, are present all around. RF/MW sources are a necessity in daily life, but they also cause a growing concern regarding the possible biological effects of microwaves. Until now, most published studies have produced conflicting and inconclusive results with respect to the effects of microwaves on human health (1). For this reason, it is important that the biological effects of RF/MW fields are understood, at least at the level of their clinical significance, so that health hazard can be assessed. Because the potential impact of RF/MW fields on human health has not yet been well characterized, the basic knowledge from laboratory studies based on cellular and animal test systems are of invaluable significance. The research of *in vivo* biological markers of RF/MW radiation has shown a clear impact on the growth, development and maturation of haematopoietic cells (2-6). *In vitro* biological effects were also reported for low frequency RF/MW fields. Changes were observed in the cell cycle, cell growth rate, enzyme activity, cell membrane structure, morphology and gene expression (7-14), including chromosome damage and apoptosis (15, 16). Non-thermal biological effects are measurable changes in biological systems that may or may not be associated with adverse health effects. However, the results from different studies are still inconclusive and conflicting.

*Preliminary report presented at the 6th Symposium of the Croatian Radiation Protection Association with international participation, Stubičke Toplice, Croatia, 18-20 April 2005

Seeing that data on biological effects of mobile phone frequencies on established cell culture lines are often contradictory our study was aimed at evaluating the influence of 864 MHz and 935 MHz electromagnetic fields on basic growth parameters of V79 cells, that is, the lung fibroblasts of the Chinese hamster.

MATERIALS AND METHODS

Cell Culture

Cell line V79 (lung fibroblasts of the Chinese hamster) was routinely cultured in a nutrient medium (RPMI 1640 medium, SIGMA Chemical CO, St. Louis, USA) supplemented with 10 % inactivated foetal calf serum (FCS, SIGMA Chemical CO, St. Louis, USA) and antibiotics. The treated and matching control cell samples were routinely grown in controlled environment at 37 °C, 5 % CO_2 and high humidity at pH 7.2.

Exposure conditions

The strength of the 864 MHz electric field was (7.3±3)V m^{-1} and of the 935 MHz electric field (8.2±0.3)V m^{-1}. In this study we used the Transversal Electromagnetic Mode Cell (TEM-cell) with a signal generator PHILIPS PM 5508 and amplifier to generate the electromagnetic field with the frequency of 864 MHz (17). The 935 MHz electromagnetic field was generated in a certified Gigahertz Transversal Electromagnetic Mode Cell (GTEM-cell) model 5402, ETS™ Lindgren, USA, under the license issued by Asea Brown Boveri of Baden Switzerland (18). The GTEM was equipped with a signal generator Hewlett Packard HP8657A.

Prepared cell samples were exposed in triplicate for one, two and three hours, both to the 864 MHz and to the 935 MHz RF/MW field frequency. Treated samples were matched with sham-exposed controls which were kept in the same experimental conditions, except that the RF/MW generator was switched off for control samples.

The specific absorption rate (SAR), that is, the rate at which energy is absorbed in biological material, averaged for a single cell, was 0.08 W kg^{-1} at 864 MHz and 0.12 W kg^{-1} at 935 MHz (19, 20). SAR was calculated by averaging the individual parameters of the cell substance in accordance with their volume fraction in the live cell according to the Steffensen's mathematical model. For example, for proteins, chromatin and extracellular medium the volume fraction of a cytoplasm is 46.3 % plus the conductivity of 1.03 S m^{-1}; nucleus volume fraction is 36.9 % plus the conductivity of 0.76 S m^{-1} (19).

In vitro tests; cell growth, colony-forming ability, and cell viability

To determine the cell growth, V79 cells were plated in a concentration of $1x10^4$ mL^{-1}. The growth curve was determined by cell counts for each hour of exposure on post-exposure days 1, 2, 3, 4, and 5. To determine colony-forming ability, 200 cells were plated on Petri dishes. Cell samples were irradiated for one, two, and three hours, and cultivated for seven days. Thereafter, newly formed colonies were stained with Giemsa dye and counted using a light microscope (10x magnification). Trypan blue exclusion test was used to determine cell viability after one, two, and three hours of microwave exposure. The viability assessment was based on the ratio of viable and nonviable cells counted over the five consecutive days (21). Analysis of variance (ANOVA/MANOVA) was used to evaluate statistical data.

RESULTS

Figures 1, 2, and 3 show the growth curves of the sham-exposed controls and V79 cell culture after one, two, and three hours of 864 MHz and 935 MHz microwave irradiation. Samples exposed to 864 MHz

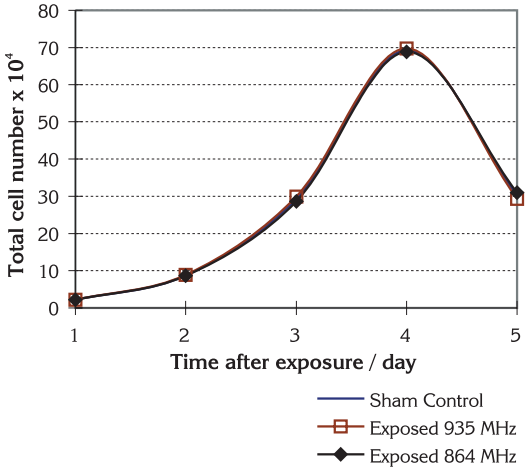

Figure 1 *Growth curve of sham-exposed controls and of V79 cell cultures exposed to 864 MHz and 935 MHz microwaves for one hour*

for two and three hours significantly differed from the sham-exposed control samples. A significant decrease in the cell number was observed on Day 3 after microwave exposure ($p<0.05$). Cell culture samples that were exposed to 935 MHz showed a similar decrease in the cell number. Significant difference was also found between cultures exposed for three hours and the sham-exposed control cultures (Figure 3). The cell population doubling time did not differ between the groups throughout the experiment, and it was 16.2 hours.

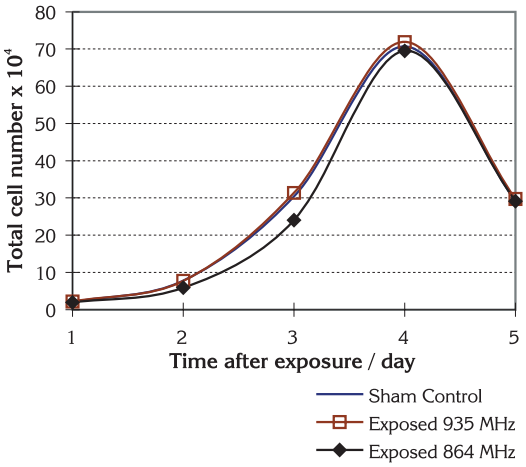

Figure 2 *Growth curve of sham-exposed controls and of V79 cell cultures exposed to 864 MHz and 935 MHz microwaves for two hours*

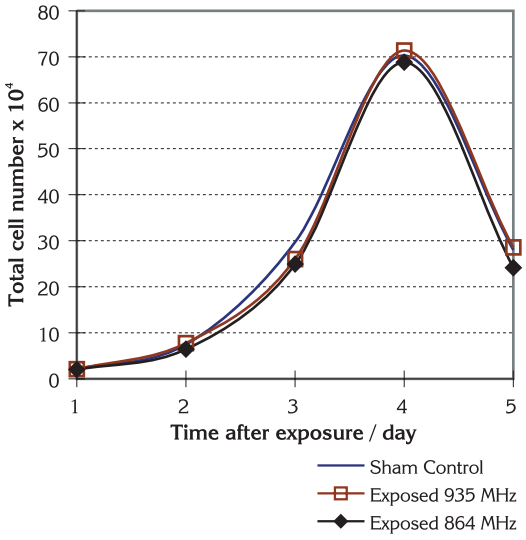

Figure 3 *Growth curve of sham-exposed controls and of V79 cell cultures exposed to 864 MHz and 935 MHz microwaves for three hours*

Figure 4 shows the colony-forming ability of V79 cells exposed to 864 MHz or 935 MHz fields for one, two and three hours and sham-exposed controls. Colony-forming ability did not significantly differ between the sham-exposed and irradiated cell culture samples, regardless of the field frequency or exposure time.

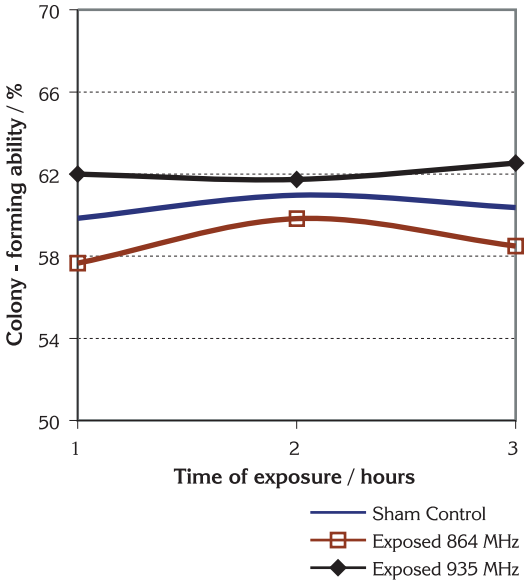

Figure 4 *Colony-forming ability of sham-exposed controls and of V79 cell cultures exposed to 864 MHz and 935 MHz microwaves for one, two or three hours*

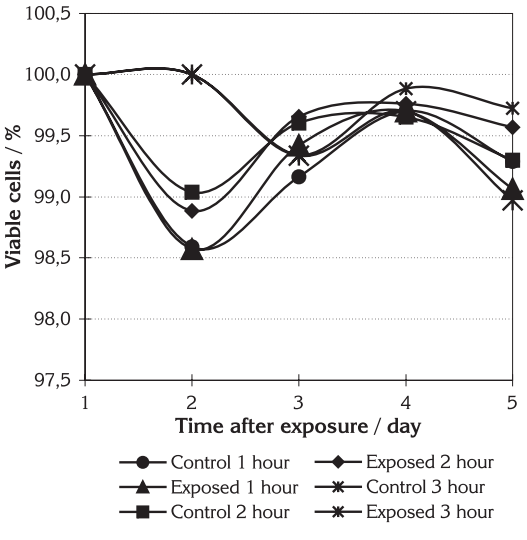

Figure 5 *Cell viability of sham-exposed controls and of V79 cell cultures exposed to 864 MHz microwaves for one, two or three hours*

Figures 5 and 6 show the viability of V79 cells exposed to 864 MHz and 935 MHz fields for one, two and three hours. The applied microwave radiation frequency of 864 MHz did not affect cell viability, ranging from 98.5 % to 100 % in both the exposed and sham control cells (Figure 5). Following exposure to 935 MHz microwave irradiation, cell viability ranged from 97 % to 100 % for all samples (Figure 6). Each data point in the curve represents the mean value obtained from six separate samples for both microwave and sham-exposed cell cultures.

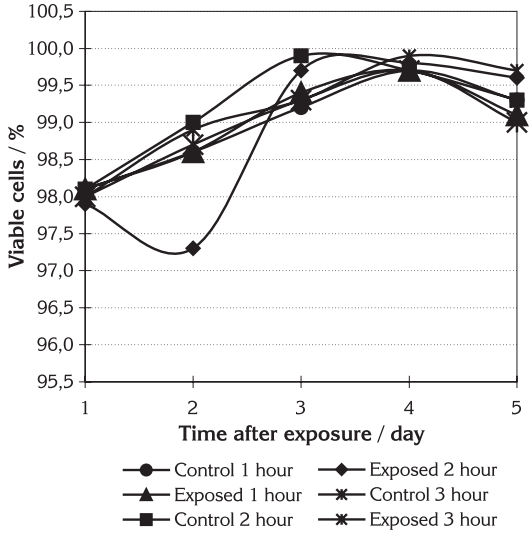

Figure 6 *Cell viability of sham-exposed controls and of V79 cell cultures exposed to 935 MHz microwaves for one, two or three hours*

DISCUSSION

The results obtained from exposing the Chinese hamster fibroblast cells to 864 MHz and 935 MHz RF/MW fields are in agreement with the findings published by *Kwee and Rasmark*, who found growth suppression of human epithelial amnion cells exposed to 960 MHz frequency field within the SAR value range of 0.021 W kg^{-1} to 2.1 W kg^{-1} (22). The same authors assumed that there was a so-called "window" effect, that is, that the maximum effect on cell proliferation rate that was related to a specific electromagnetic field and exposure time. These effects were not linear over the whole radiofrequency spectrum. The non-linear effect could be explained by oscillations in cell growth (23). *Velizarov et al*. also reported a significant change in cell proliferation in RF/MW-exposed, transformed human epithelial amnion cells. The experiment was conducted at the temperature of 39 °C or 35 °C in order to separate thermal from non-thermal RF/MW effect on cell proliferation. The authors attributed altered cell proliferation to exposure to electromagnetic field, and not to the influence of temperature (24). *French et al*. found a significant growth inhibition of human astrocytoma cells caused by a 835 MHz RF/MW field whose density was 8.1 mW cm^{-2} (25). Otherwise, no changes in the cycle progression of mouse fibroblasts or human glioma cells were observed following exposure to 835.62 MHz or 847.44 MHz fields at the average specific absorption rate of 0.6 W kg^{-1}. In a similar study by *Stagg et al*. which involved the exposure of rat glioma cells and primary rat glial cells to a 836.55 MHz field, no changes were observed in the growth curve of these cell lines (26, 27). This study has demonstrated that the applied RF/MW frequencies affect V79 cell proliferation. A transient decrease in the total cell number and preserved viability and colony-forming ability of cells, regardless of exposure duration to RF/MW fields suggest that the applied RF/MW frequencies affect the V79 cell growth and development almost identically.

Acknowledgement

This study was supported by grant No. 0022005 of the Ministry of Science, Education and Sports of the Republic of Croatia.

REFERENCES

1. Elwood JM. Epidemiological studies of radio frequency exposures and human cancer. Bioelectromagnetics 2003;24(Suppl 6):63-73.
2. Trošić I, Bušljeta I, Pavičić I. Blood-forming system in rats after whole-body microwave exposure: reference to the lymphocytes. Toxicol Lett 2004;154:125-32.
3. Trošić I, Bušljeta I, Modlic B. Investigation of the genotoxic effect of microwave irradiation in rat bone marrow cells: in vivo exposure. Mutagenesis 2004;19:361-4.
4. Trošić I, Bušljeta I, Kašuba V, Rozgaj R. Micronucleus induction after whole-body microwave irradiation of rats. Mutat Res 2002;521:73-9.
5. Trošić I, Bušljeta I. Frequency of micronucleated erythrocytes in the rat bone marrow exposed to 2.45 GHz radiation. Physica Scripta 2005;T118:168-70.
6. Bušljeta I, Trošić I, Milković-Kraus S. Erythropoietic changes in rats after 2.45 GHz nonthermal irradiation. Int J Hyg Environ Health 2004;207:1-6.

7. Cleary SF, Cao GH, Liu LM: Effect of isothermal 2.45 GHz microwave radiation on the mammalian cell cycle: comparison with effects of isothermal 27 MHz radiofrequency radiation exposure. Bioelectrochem Bioenerg 1996;39:167-73.
8. Grundler W, Keilmann F. Nonthermal effects of millimeter microwaves on yeast growth. Z Naturforsch 1978;33:15-22.
9. Dutta SK, Das K, Gosh B, Blackman CF. Dose dependence of acetylcholinesterase activity in neuroblastoma cells exposed to modulated radio-frequency electromagnetic radiation. Bioelectromagnetics 1992;13:317-22.
10. Pentafield LM, Litovitz T, Krause D, Desta A, Mullins JM. Role of moudlation on the effect of microwaves on ornithine decarboxylase activity in L929 cells. Bioelectromagnetics 1997;18:132-41.
11. Phelan AM, Lange DG, Kues HA, Lutty GA. Modification of fluidity in melanin-containing cells by low level microwave radiations: Bioelectromagnetics 1992;12:131-46.
12. Pacini S, Ruggiero M, Iacopo S, Aterini S, Gulisano F, Gulisano M. Exposure to global system for mobile communication (GSM) cellular phone radiofrequency alters gene expression, proliferation, and morphology of human skin fibroblasts. Oncol Res Anticancer Drug Des 2002;13:19-24.
13. Fritze K, Wiessner C, Kuster N, Sommer C, Gass P, Hermann DM, Klessing M, Hossmann KA. Effect of GSM microwave exposure on the genomic response of the rat brain. Neuroscience 1997;81:627-39.
14. Goswami PC, Albee LD, Parsian AJ, Baty JD, Moros EG, Pickard WF, Roti Roti JL, Hunt CR. Proto-oncogene mRNA levels and activities of multiple transcription factors in C3H 10 T ´ murine embryonic fibroblasts exposed to 835.62 and 847.74 MHz cellular phone communication frequency radiation. Radiat Res 1999;151:300-9.
15. Zeni O, Chiavoni AS, Sannino A, Antolini A, Forgio D, Bersani F. Lack of genotoxic effects (micronucleus induction) in human lymphocytes exposed In vitro to 900 MHz electromagnetic fields. Radiat Res 2003;160:152-8.
16. Peinnequin A, Piriou A, Matheiu J, Dabouis V, Sdebbah C, Malabiau R, Debouzy JC. Non-thermal effects of continuous 2.45 GHz microwave on Fas-induced apoptosis in human Jurkat T-cell line. Bioelectrochemistry 2000;51:157-61.
17. Malaric K, Bartolic J. Design of a TEM-cell with Increased Usable Test Area. Turk J Electr Engin Comp Sci 2003;112:143-54.
18. Operational Manual Gigahertz Transverse Elelctromagneic cell (GTEM). Model 5400 Series. Austin, Texas: EMC Test Systems, LP - EMCO; 1999.
19. Steffensen KV, Raskmark P, Pedersen GF. FDTD calculations of the EM-field distribution in a microtiter suspension well. In: Proceedings of the COST 244: Biomedical Effects of Electromagnetic fields; 3-4 Sep 1995; Kuopio, Finland. 1995. p.80-7.
20 WHO. Environmental Health Criteria 137: Electromagnetic Fields (300 Hz to 300 GHz). Geneva: World Health Organization; 1993. p.79.
21. Freshney RI. 2000. Culture of animal cells: A manual of basic technique. 4th ed. New York (NY): Wiley-Liss; 2000.
22. Kwee S, Rasmark P. Changes in cell proliferation due to environmental non-ionizing radiation 2. Microwave radiation. Bioelectrochem Bioenerg 1998;44:251-5.
23. Grundler W, Kaiser F. Experimental evidence for coherent excitations correlated with cell growth. Nanobiology 1992;1:163-76.
24. Velizarov S, Rasmark P, Kwee S. The effects of radiofrequency fields on cell proliferation are non-thermal. Bioelectrochem Bioenerg 1999;48:171-80.
25. French PW, Donnellan M, McKenzie DR. Electromagnetic radiation at 835 MHz changes morphology and inhibits proliferation of a human astrocytoma cell line. Bioelectrochem Bioenerg 1997;43:13-8.
26. Stagg RB, Thomas WJ, Jones RA, Adey WR. DNA synthesis and cell proliferation in C6 glioma and primary glial cells exposed to a 836.55 MHz modulated radiofrequency field. Bioelectromagnetics 1997;18:230-6.
27. Higashikubo R, Ragouzis M, Moros EG, Straube WL, Roti Roti JL. Radiofrequency electromagnetic fields do not alter the cell cycle progression of C3H 10T1/2 and U87MG cells. Radiat Res 2000;156:786-95.

Sažetak

USPOREDBA DJELOVANJA MIKROVALNOG ZRAČENJA FREKVENCIJA 864 MHz I 935 MHz NA STANICE U KULTURI

Cilj istraživanja bio je usporediti utjecaj 864 MHz i 935 MHz mikrovalnog zračenja na stanični rast, sposobnost stvaranja kolonija (CFA) i vijabilnost trajne kulture V79-stanica. Prethodno pripremljeni uzorci stanične kulture izlagani su 1, 2 i 3 sata određenim mikrovalnim poljima. Za frekvenciju 864 MHz upotrijebljena je transverzna elektromagnetska komora (TEM komora) opremljena generatorom i pojačalom signala. Polje frekvencije 935 MHz stvoreno je s pomoću gigahercne transverzne elektromagnetske komore (GTEM komora) i generatora signala. Prosječna vrijednost specifične brzine apsorpcije (SAR) iznosila je 0.08 W kg^{-1} za 864 MHz te 0.12 W kg^{-1} za 935 MHz. Stanice su rasle u standardnim laboratorijskim uvjetima (>80 % vlage, 37 °C, 5 % CO_2). Za određivanje krivulje staničnog rasta V79-stanice su nasađene u koncentraciji od $1x10^4$ mL^{-1} hranjivog medija. Iz početne koncentracije od 200 nasađenih stanica sedam dana nakon ozračivanja određena je CFA za svako vrijeme izloženosti brojem nastalih staničnih kolonija. Vijabilnost stanica određena je nakon 1, 2 i 3 sata izloženosti RF/MW poljima s pomoću tripanskog plavila. Vijabilnost je utvrđena omjerom živih i mrtvih stanica tijekom 5 uzastopnih dana pokusa.
Rast stanica izloženih 2 i 3 sata polju frekvencije 864 MHz bio je značajno inhibiran treći dan nakon izlaganja, a polje frekvencije 935 MHz je u trosatnoj izloženosti uzoraka uzrokovalo značajan pad broja stanica samo trećeg dana. CFA i vijabilnost stanica izloženih poljima od 864 MHz i 935 MHz nisu se značajno razlikovale od odgovarajućih sham izloženih uzoraka.
Mikrovalna polja spomenutih osobina djelovala su na gotovo istovjetan način na rast stanične kulture, sposobnost stvaranja kolonija i vijabilnost V79-stanica.

KLJUČNE RIJEČI: *GTEM komora, izloženost mikrovalovima, sposobnost stvaranja kolonija, stanični rast, TEM komora, V79-stanice, vijabilnost*

REQUESTS FOR REPRINTS:

Ivan Pavičić, M.Sc.
Institute for Medical Research and Occupational Health
P.O. Box 291, HR-10001 Zagreb, Croatia
E-mail: *ipavicic@imi.hr*