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IMPACT OF 864 MHZ OR 935 MHZ RADIOFREQUENCY MICROWAVE RADIATION ON THE BASIC GROWTH PARAMETERS OF V79 CELL LINE

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The aim of this study was to evaluate and compare the influence of 864 MHz and 935 MHz radiofrequency/microwave (RF/MW) fields on the growth, colony-forming ability, and viability of V79 cells (continuous line). Cell samples with 1×10^4 V79 cells each, were exposed to continuous wave frequencies of 864 MHz and 935 MHz for 1, 2 and 3 hours. Exposed samples were matched with unexposed control samples. Specific absorption rate (SAR) was 0.08 W/kg for the 864 MHz or 0.12 W/kg for the 935 MHz field. Cell growth and viability were determined by counting cells every day for five days after exposure. Colony-forming ability was assessed by counting colonies seven days after exposure. The growth of the 864 MHz-irradiated cells was significant after two- and three-hour exposure 72 hours after irradiation ($p < 0.05$). The similar was observed 72 hours after exposure for cells exposed to 935 MHz microwaves for three hours ($p < 0.05$). Colony-forming ability and cell viability in V79 cells exposed to 864 MHz or 935 MHz microwaves did not significantly differ from control cells. The two applied RF/MW fields showed similar effects on the growth, colony-forming ability and viability of V79 cells. Cell growth impact was time-dependent for both fields.

Keywords: V79 cells – exposure – 864 MHz – 935 MHz – growth – colony-forming ability – viability

INTRODUCTION

Sources of radiofrequency/microwave (RF/MW) radiation, particularly mobile phones, are present all around us. RF/MW sources are a necessity of everyday life, but they also raise concern about biological hazards involved. Microwave exposure studies have produced conflicting and inconclusive results about the effects of microwaves on human health [4]. For this reason, it is important that the biological

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effects of RF/MW fields, if any, are proven and understood, at least at the experimental level of significance, using *in vitro* models. Fundamental knowledge based on cellular and animal test systems is invaluable in elucidating uncertainties about RF/MW influence on the living matter. Reported *in vivo* biological markers of RF/MW radiation have shown influence on the growth, development and maturation of rats' haematopoietic cells during whole-body exposure [1, 25–29]. *In vitro* biological effects were also reported for low-level RF/MW frequency fields. These revealed changes in the cell cycle, cell growth rate, enzyme activity, cell membrane structure, morphology and gene expression [2, 3, 9, 10, 12, 18, 20, 22], as well as chromosome damage and apoptosis [21, 32]. Since biological effects of low-level RF/MW fields are non-thermal, measurable changes in biological systems may not be necessarily associated with the harmful impact on physiological processes. The aim of this study was to evaluate and compare the effects of 864 MHz and 935 MHz microwave fields on the basic growth parameters of V79 cells, that is, growth, colony-forming ability and cell viability.

MATERIALS AND METHODS

Cell culture

A continuous line of V79 cells, i.e. 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% heat inactivated foetal calf serum (FCS, SIGMA Chemical CO, St. Louis, USA) and antibiotics. The exposed and control cell samples were routinely grown in controlled conditions, in a highly humid atmosphere with 95% air and 5% CO_2, at pH 7.2 and temperature of 37 °C.

Exposure conditions

To generate an electromagnetic field of 864 MHz, we used a Transversal Electromagnetic Mode Cell (TEM-cell) with a signal generator Philips PM 5508 and an amplifier [15]. The 935 MHz electromagnetic field was generated using 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 [17]. The GTEM-cell was equipped with a signal generator Hewlett Packard HP8657A. The power density of the 864 MHz electric field was 0.14 ± 0.024 W/m^2 and of the 935 MHz electric field 0.17 ± 0.002 W/m^2. The temperature of the medium was measured for all three hours of irradiation and remained 37 ± 0.5 °C.

Prepared cell samples were exposed in triplicate for one, two and three hours, both to the 864 MHz and 935 MHz RF/MW field. Control samples were kept in the same experimental conditions, but were not exposed to microwaves.

Using the equation of the World Health Organisation, we calculated that the average specific absorption rate (SAR) for a single cell was 0.08 W/kg at 864 MHz and 0.12 W/kg at 935 MHz [31]. SAR was calculated by averaging individual cell content including proteins, water, nucleic acids etc., in accordance with their volume fraction [24].

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

To determine the cell growth, V79 cells were plated on 24 well plates (Tissue Culture Testplate, TPP, Switzerland) with a 1×10^4 cells per 1 millilitre of nutrient medium for each well. According to Freshney's protocol, the growth curve was determined by cell counts for each hour of exposure on post-irradiation hours 24, 48, 72, 96 and 120 [6]. To determine colony-forming ability, 200 cells in 5 millilitres were plated on 10 cm diameter Petri dishes (TPP, Switzerland). 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 (×10 magn.) [7]. 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 five consecutive days [8].

Statistical analysis

Collected data were analysed using Statistica 7.0 (StatSoft Inc., USA). Results were presented as mean values and standard deviations or errors (SD/SE). Pair wise comparisons among the groups of data were performed using analysis of variance ANOVA/MANOVA. Significant differences were set at $p < 0.05$.

RESULTS

Table 1 and Figures 1, 2 and 3 show the growth curves of control V79 cells and cells exposed for one, two and three hours to 864 MHz or 935 MHz microwave irradiation. Samples exposed to 864 MHz for two and three hours differed from control samples. A significant decrease in the cell number was observed 72 hours after 864 MHz microwave exposure ($p < 0.05$). Cells exposed to 935 MHz showed a similar significant decrease in the cell number. A significant difference was also found between cultures exposed for three hours and control cultures (Fig. 3 and Table 1). The cell population doubling time did not differ between the groups throughout the experiment, and it was 16.2 hours.

Figure 4 shows the colony-forming ability of V79 cells exposed to the 864 MHz or 935 MHz fields for one, two and three hours and of control cells. Colony-forming

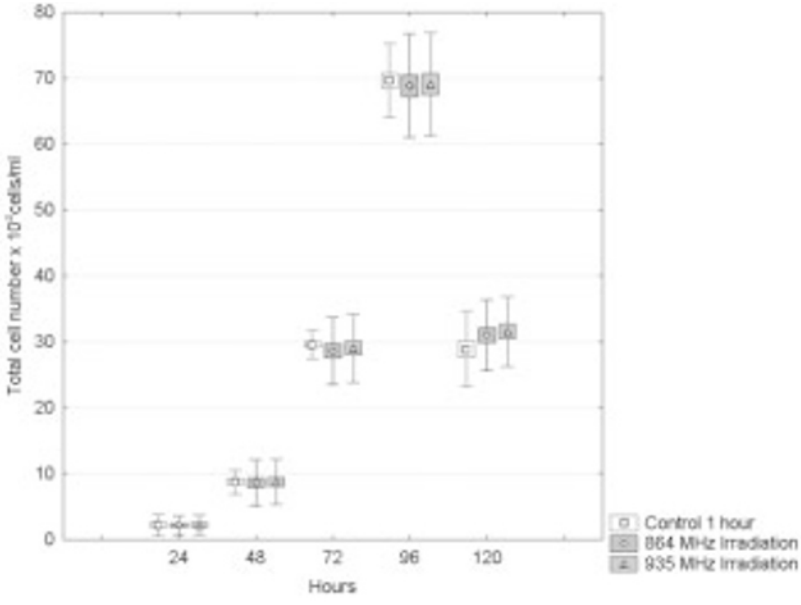

Fig. 1. Growth curve of control V79 cell cultures and those exposed to 864 MHz and 935 MHz microwaves for one hour

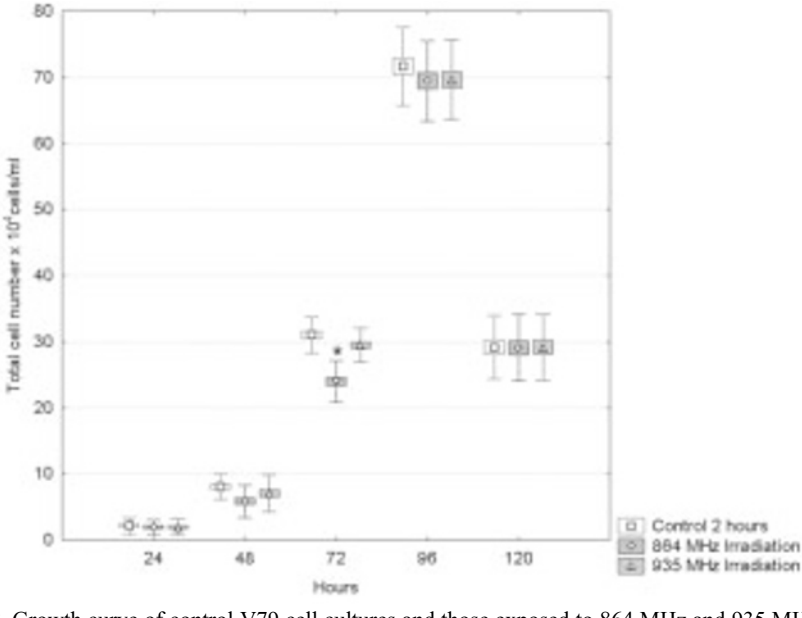

Fig. 2. Growth curve of control V79 cell cultures and those exposed to 864 MHz and 935 MHz microwaves for two hours (*significant difference $p < 0.05$)

Table 1
Cell growth descriptive statistics of control V79 cell cultures and those exposed to 864 MHz and 935 MHz fields for one, two and three hours

Time	Hours of exposure	Control cultures	935 MHz exposure	864 MHz exposure
		X cell number ±SD (*10^4)		
24 hours	1	2.2 ± 1.5	2.2 ± 1.5	2.2 ± 1.5
	2	2.2 ± 1.3	2.0 ± 1.2	2.0 ± 1.1
	3	2.1 ± 1.5	2.1 ± 1.3	2.0 ± 1.2
48 hours	1	8.8 ± 1.9	8.8 ± 3.5	8.6 ± 3.5
	2	8.2 ± 2.7	7.1 ± 2.8	5.9 ± 2.5
	3	7.7 ± 1.9	7.3 ± 2.3	6.5 ± 1.8
72 hours	1	29.3 ± 4.3	29.0 ± 5.2	28.6 ± 5.1
	2	31.3 ± 3.9	29.5 ± 2.6	24.0 ± 3.2*
	3	31.4 ± 4.0	28.1 ± 3.2*	25.0 ± 3.7*
96 hours	1	69.3 ± 6.7	69.1 ± 7.8	68.9 ± 7.8
	2	71.8 ± 3.1	69.6 ± 6.1	69.5 ± 6.0
	3	71.3 ± 2.4	69.3 ± 5.5	68.9 ± 5.5
120 Hours	1	29.1 ± 5.8	31.5 ± 5.2	31.0 ± 5.4
	2	29.4 ± 4.9	29.0 ± 4.9	29.1 ± 5.0
	3	29.2 ± 5.3	26.1 ± 4.0	24.1 ± 4.6

* Significant difference $p < 0.05$.

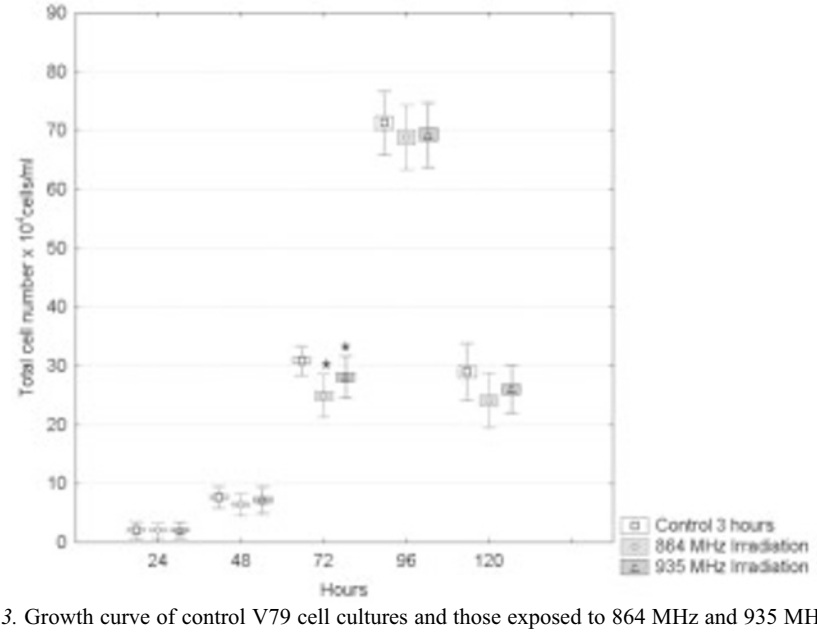

Fig. 3. Growth curve of control V79 cell cultures and those exposed to 864 MHz and 935 MHz microwaves for three hours (*significant difference $p < 0.05$)

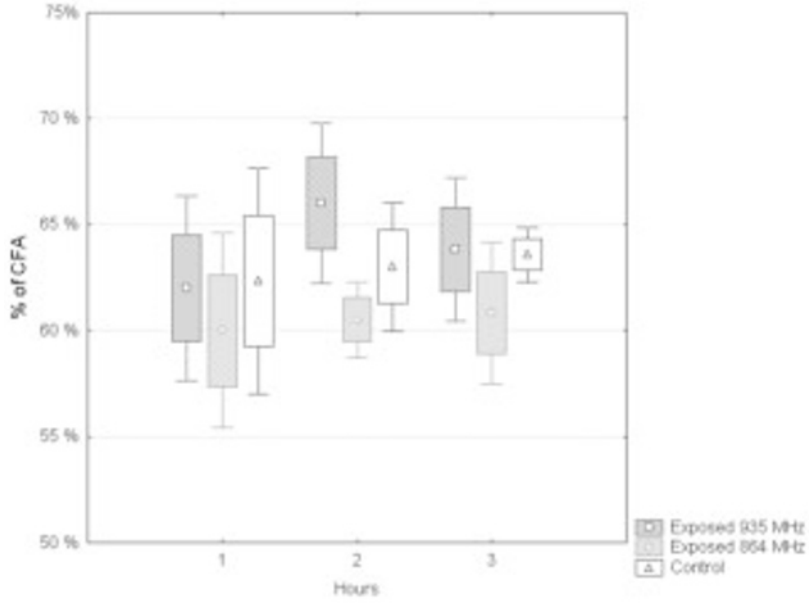

Fig. 4. Colony-forming ability of control V79 cell cultures and those exposed to 864 MHz and 935 MHz microwaves for one, two or three hours

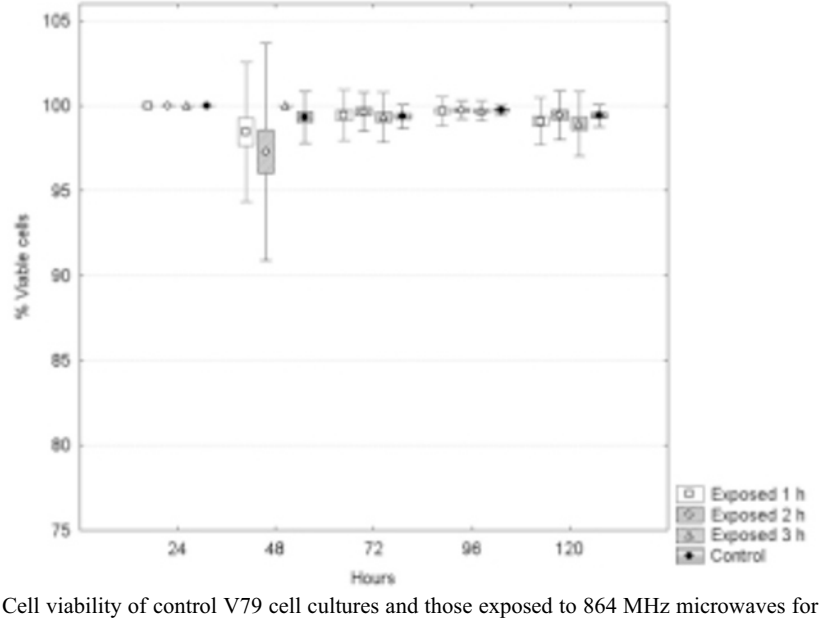

Fig. 5. Cell viability of control V79 cell cultures and those exposed to 864 MHz microwaves for one, two or three hours

ability did not significantly differ between the unexposed and exposed cells, regardless of the field frequency or exposure time.

Figures 5 and 6 show the viability of V79 cells irradiated with 864 MHz and 935 MHz fields for one, two and three hours. The 864 MHz field did not affect cell viability, which ranged from 98.5% to 100% in both exposed and control cells (Fig. 5). Following exposure to the 935 MHz microwave irradiation, cell viability ranged from 97% to 100% for all samples (Fig. 6). Each data point in the curve represents the mean value and associated standard deviation or error (SD/SE) obtained from six separate samples.

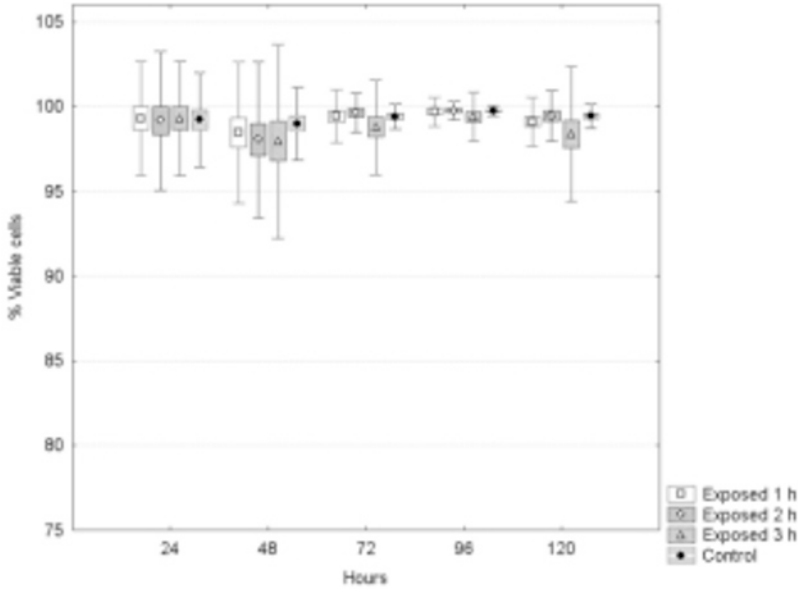

Fig. 6. Cell viability of control V79 cell cultures and those exposed to 935 MHz microwaves for one, two or three hours

DISCUSSION

This study showed cell growth decrease in V79 cells 72 hours after two- and three-hour exposure to 864 MHz microwave field with SAR 0.08 W/kg. In the same way, cells exposed to 935 MHz microwaves at SAR of 0.12 W/kg for three hours showed a significantly lower growth 72 hours after exposure ($p < 0.05$) (Figs 2, 3, Table 1). Colony-forming ability and cell viability did not significantly differ between the exposed and control cells. Our results are in accordance with the findings published by Kwee and Rasmark, who found growth suppression of human epithelial amnion cells exposed to 960 MHz frequency field within SAR ranging between 0.021 W/kg and 2.1 W/kg [14]. The same authors pointed to a so-called "window" effect, that is,

the maximum effect on cell proliferation rate was related to a specific electromagnetic field and to exposure time. This means that these effects were not linear over the whole radiofrequency spectrum. The non-linear effect could be clarified by the findings of Grundler and Kaiser, who reported that microwave irradiation, could affect the growth rate of yeast cells in a frequency-selective manner. Depending on frequency, both increase and decrease of the growth rate were described. Furthermore, the resonance bandwidths were found to be of the order of 10 MHz. In this way, authors preserved theoretical predictions of coherent molecular oscillations which could activate cellular metabolic processes [11]. Recently published results showed that an external electric field in the similar frequency range dissipated essential cellular proteins, constituting the microtubules of the cytoskeleton [19]. Inasmuch as the fundamental structure such as microtubule proteins is disturbed by irradiation, it is reasonable to assume that this, in turn, could affect the cell growth.

Velizarov et al. [30] also reported a significant change in cell proliferation in RF/MW-exposed, transformed human epithelial amnion cells. The experiment was conducted at 39 °C or 35 °C in order to separate thermal from non-thermal RF/MW effects on cell proliferation. The authors attributed altered cell proliferation to electromagnetic field exposure, but not to the influence of temperature [30]. Additionally, French et al. observed a significant growth inhibition of human astrocytoma cells by 835 MHz field whose density was 8.1 mW/cm [5]. Otherwise, no changes in the cycle progression of mouse fibroblasts or human glioma cells were reported following exposure to 835.62 MHz or 847.44 MHz fields at the average SAR of 0.6 W/kg. In similar studies by Higashikubo et al. and Stagg et al., which involved the exposure of rat glioma cells and primary rat glial cells to an 836.55 MHz field, no changes were observed in the growth curve of these cell lines [13, 23]. Negative findings were also reported by Miyakoshi et al. [16]. This group of authors found that 1950 MHz fields with SARs ranging from 1 to 10 W/kg did not affect the growth of human glioma cells. However, they also reported that exposure to a RF/MW field with SAR of 10 W/kg for 1 and 2 hours significantly decreased the protein level of phosphorylated heat shock protein (Hsp 27) [16].

Our RF/MW fields showed a similar effect on the growth, colony-forming ability and viability of V79 cells. Cell growth findings revealed a time-dependent effect of both 864 MHz and 935 MHz fields. The observed drop in the cell growth is considered temporary because additional cytotoxic parameters, i.e. viability and colony-forming ability remained unchanged throughout the experiment. As our results suggest that microwave radiation affects the cell growth kinetics in a time-dependent manner, further research should include longer exposure time(s).

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