

Microwave Irradiation Influences on the State of Human Cell Nuclei

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Changes of electrokinetic properties of cell nuclei and the quantity of granules of heterochromatin located near the nuclear envelope in nuclei of human buccal epithelium cells were studied under the influence of electromagnetic fields in vitro. Irradiation of cells was realized by means of a semiconductor generator of millimeter radiation (wavelength 7.1 mm, frequency 42.2 GHz), the Yav-1 apparatus for extremely high frequency therapy. It was shown that irradiation of cells induced a decrease in electric charge of native human buccal epithelium cell nuclei and an increase in chromatin condensation in nuclei. The observed effects depend on irradiation dose and individual peculiarities of donors. Bioelectromagnetics 19:414–419, 1998. © 1998 Wiley-Liss, Inc.

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INTRODUCTION

Microwave radiation is a factor that affects many parameters of the functional state of the human organism. On the one hand, it may present a threat to health [Bernhardt, 1992; Hester, 1992], and on the other, it may be used in medical treatment [Bassett, 1993].

Low-intensity microwave irradiation influences various aspects of a cell's life: the properties of cell membranes [Wachtel et al., 1975; Liburdy et al., 1988; Somosy et al., 1991], transcription activity [Byus et al., 1988], and the puffing pattern in polytene chromosomes [Koschnitzke et al., 1983]. A direct mutagenic effect of microwaves was not observed by some authors [Michaelson, 1980; Marec et al., 1985], but was by others [Garaj-Vrhovac et al., 1990]. A microwave-induced increase in percentage of cells with chromosome aberrations and decrease of DNA synthesis was observed in cultured animal cells [Garaj-Vrhovac et al., 1990]. In an overview, [Michaelson, 1980], based on experimental data, the conclusion was drawn that microwave irradiation of intensity less than 10 mW/cm² has no mutagenic effect, and, in the interpretation of experimental results based on analysis of chromosome aberrations the action of different external factors must be taken into consideration. The first of these is the thermal effects of microwave irradiation.

The cell nucleus is the center that integrates reactions of the cell to the external factors. An important role in these processes is played by supramolecular

interactions of the nuclear components that result in changes of biophysical properties of nuclei. In this connection, studies of integral indices of the nuclear state under the influence of electromagnetic irradiation are of special interest.

In the present work, we investigated the influence of microwave irradiation on two aspects of the cell nucleus. We studied the electrophoretic properties of cell nuclei using an intracellular microelectrophoresis method developed in the Department of Genetics and Cytology of Kharkov State University [Shakhbazov et al., 1985], as well as chromatin condensation in interphase nuclei, using orcein staining of heterochromatin. We also studied cell membrane integrity, using vital indigo carmine staining. The purpose of this work was the further elucidation of the role of cell nucleus and cell membrane integrity in cell reactions to electromagnetic irradiation.

The electrokinetic properties of cell nuclei are connected with the processes of regulation of the functional activity of cell nuclei. An increase in the electrophoretic mobility of nuclei isolated from mammalian cells was demonstrated in the course of activation of nuclei connected with the process of regeneration [Chaudhuri and Lieberman, 1965; Maekawa, 1967],

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cell carcinogenic transformation [Maekawa, 1967], and transfer of cells in culture [Kishimoto and Lieberman, 1965].

In contrast to the investigations mentioned above, we used the intracellular microelectrophoresis method. This technique gives no opportunity to determine the value of the electrokinetic potential because it is very difficult to determine correctly the viscosity and electric conductivity of cytoplasm, and these parameters are very unsteady. On the other hand, the intracellular microelectrophoresis method has some advantages, because, in the process of isolation of cell nuclei their properties change. In particular, the molecular weight of DNA decreases and physical properties of chromatin and its template activity change [Nicolini et al., 1976]. It is agreed that the cell nucleus is structurally and energetically connected with the cytoplasm and cell organelles. Isolation of cell nuclei from cells, by destroying the cell and centrifugation, naturally destroys these connections and changes the state of nucleus. That is why, in the Department of Genetics and Cytology of Kharkov State University, the intracellular microelectrophoresis of cell nuclei method was developed.

In the many investigations that used the intracellular microelectrophoresis method, it was shown that the electric charge of nuclei increases under the influence of phytohormones and decreases after heat shock and other injurious agents in the cells of plants [Shakhbazov et al., 1989; Shckorbatov and Shakhbazov, 1992]. In human cells, this characteristic changes in relation to age and to the state of health of donors [Shakhbazov et al., 1985, Shckorbatov and Shakhbazov, 1992].

Heterochromatin is the part of the chromatin that retains the property of intense staining through interphase; it is genetically inactive and undergoes DNA replication generally later in the cell cycle than euchromatin. Heterochromatic chromosome segments often appear as more condensed chromocenters. Heterochromatic can be divided into two categories, constitutive and facultative. Constitutive heterochromatin is never transcribed. Facultative heterochromatin is active in other cell types or even in other chromosomes within the cell, and it possesses functional genes. The main function of the facultative heterochromatin may be regulation of gene action, since the heterochromatinized segments are inactivated. Both constitutive and facultative heterochromatin, in most cases, are attached to the nuclear membrane [Therman and Susman, 1993].

Summing up, both characteristics—the electrokinetic properties of cell nuclei and the state of heterochromatin—are connected to the functional activity of nuclei and their parallel investigation may be useful in

studies of the mechanisms of the influence of external factors on cells.

MATERIALS AND METHODS

Our experiments were performed on human buccal epithelium cells of female donors of different ages. Cells were lightly scraped with a sterile blunt spatula from the internal surface of the cheek of the donor. Intact cells were placed in 1 ml of a solution composed of 2.89 mM calcium chloride in 3.03 mM phosphate buffer (pH 7.0).

The buccal epithelium is regarded as a two-compartment structure. Cell differentiation occurs in the small, lower, formative compartment that contains basal and parabasal cells. Differentiated cells mature in the large, upper compartment that contains intermediate and superficial cells. Five types of cells are present in this layer, and the maximal DNA, RNA, and protein content is observed in intermediate cells with round nuclei [Bhattacharya et al., 1983]. In our experiments, we studied two types of surface layer cells: intermediate cells with round nuclei and intermediate cells with oval nuclei.

We used a semiconductor generator of millimeter radiation (wavelength 7.1 mm, $f = 42.2$ GHz), the Yav-1 apparatus for extremely high frequency therapy. No modulation of the electromagnetic wave was used, and irradiation of objects was in free space, in the nearest area (1 cm from the antenna aperture). The intact cells were placed on a cover slide and covered by an other cover slide. The space between slides containing cells was 0.1 mm. The power density of irradiation on the cover slide surface was 0.2 mW/cm². The sample of cells and two cover slides conducted 65% of the initial irradiation energy. We explored effects of low intensity of electromagnetic irradiation. Our experiments were conducted at 25 °C. Temperature measurements in the cell samples during the process of irradiation were conducted by means of an electric thermometer (PG-2; USSR). The temperature of the sample increased in the process of irradiation. Maximal temperature shift (0.9 °C) was registered after 1.5 min of irradiation; further exposure was not accompanied by a further increase in the temperature. In previous experiments, heating of cells of human buccal epithelium to 50 °C had no significant effect on the electrokinetic properties of cell nuclei [Atramentova et al., 1976]; therefore, we assumed that heating effects have no significant role in our present experiments.

Electrokinetic properties of buccal epithelium cell nuclei were investigated by applying the method of intracellular microelectrophoresis described earlier [Shakhbazov et al., 1985]. The intact cells were investi-

gated in a flat microelectrophoretic cell with 2 cm between electrodes in the solution described above. The strength of the electric field was 15 V/cm, and the electric current was 0.1 mA. We monitored the movement of nuclei within intact cells. In these conditions nuclei displaced to the anode in just some cells. The percentage of cells with negatively charged nuclei was defined as electronegativity of nuclei (ENN). In each experiment 8–10 determinations were conducted, and 100 nuclei/determination were analyzed.

The technique of investigating the stability of cell membranes by means of indigo carmine cell staining is described in the work of Shckorbatov et al. (1995). Briefly, a 4 mM solution of indigo carmine was prepared with the buffer described previously (CaO₂ in phosphate buffer). Then 25 µl of cell suspension were mixed on a slide with the same volume of stain and no fixative. The cell preparation was then covered with a cover slide. The stainability of cells by indigo carmine was examined after 5 min at $\times 300$ magnification. Following the staining procedure, only some cells became stained: 100 cells were counted in each preparation and the percentage of stained cells was determined.

Heterochromatin sites in interphase nucleus were determined with orcein staining [Darlington and La-Cour, 1976]. A 2% orcein solution in 45% acetic acid was applied; 25 µl of the cell suspension were mixed with the same volume of stain on a preparation slide for 1 h. The percent of nuclei containing granules of heterochromatin located near the nuclear envelope was defined as near-envelope heterochromatin (NEH). In each experiment ten measurements were conducted, and for each measurement 100 nuclei were analyzed.

All experimental results were statistically processed using Student's *t*-test; the mean values of eight to ten determinations and standard errors of the mean are presented in the tables.

RESULTS

According to our data, 1 microwave irradiation caused a significant decrease of ENN in cells of donors C and D (Table 1). Increasing exposure to 15 s produced a significant decrease in ENN of cells of donor A, but a further increases of exposure did not result in a further decrease of ENN. In the cells of donors B and E, a statistically significant change of ENN under the influence of microwave radiation was not detected; a tendency toward an increase of this parameter was observed in donor B.

In Table 2 gives the results of 5 s irradiation of cells of donors of different ages. These results demonstrate microwave-induced decrease of ENN.

The initial level of ENN was different in the cells

of different donors; this is related to the ages and individual peculiarities of the donors. According to previous results, obtained on more than 2000 donors in the Department of Genetics and Cytology of Kharkov State University, every age group of donors has a definite value of ENN [Shakhbazov et al., 1985]. It must be remembered that mean group values of ENN are obtained for large groups of people. In every individual case the ENN level deviates from the mean group level and these deviations (especially excess of ENN) may not be inevitably connected to abnormalities in the state of the organism. The ENN value is also influenced by factors affecting the state of an organism (tiredness, disease, etc.) [Shckorbatov and Shakhbazov, 1992]. In our experiments, the ENN of donors A, C, D, and F–N was not lower than the values of corresponding age groups (in donors A, D, F, G, J, and K–M the values were higher than age norm), but the levels of donors B and E it were significantly less than expected. In donors B and E, microwave irradiation had no pronounced effect on ENN. We can suppose that microwave irradiation lowers ENN in donors with normal or advanced level of ENN, but not in donors with decreased ENN.

We investigated the change in ENN due to 5 s irradiation, evaluated at different times (5–180 min) following irradiation (Table 3). The data presented in Table 3 were obtained in cells of donor E several months later than data presented in Table 1, and during this period the control level of ENN changed from 37.3 to 77.8% because of some physiological changes in the state of the organism. These changes may be connected to different stages of the menstrual cycle, because, as we have shown earlier, ENN significantly changes during this cycle [Shakhbazov and Colupaeva, 1991]. It was shown that the effect of irradiation in cells of donor E was practically independent of time in the period 5–45 min after irradiation. In this period, ENN remained on the same reduced level, but after 55 and 180 min ENN manifested a tendency toward a further decrease.

It is known that the level of condensation of chromatin is directly related to the level of its activity [van Holde, 1989]. The NEH parameter in our experiments reflects for the most part the quantity of heterochromatin granules representing telomeric heterochromatin (chromocenters) and Barr bodies (condensed X chromosome) [Therman and Susman, 1993]. In our experiments, it was shown that NEH increased considerably after microwave irradiation (Table 4). This finding indicates an increase in chromatin condensation. The minimal exposure (1 s) caused a significant increase in NEH in nuclei of donor B; 5 s of exposure promoted an increase in this parameter in cells of donor B and

TABLE 1. Effect of Microwave Irradiation on Electronegativity of Cell Nuclei (ENN, %) After a 1 s Irradiation (Cells monitored 5 min after irradiation)

Exposure (s)	Donor (age in yr in parentheses)				
	A (6)	B (25)	C (25)	D (36)	E (24)
Age norm	21.1 ± 3.3	71.0 ± 3.0	71.0 ± 3.0	50.9 ± 3.9	73.0 ± 3.0
0 (control)	37.8 ± 2.8	59.5 ± 2.7	70.3 ± 2.8	75.7 ± 2.7	37.3 ± 2.2
1	31.3 ± 2.7	63.7 ± 3.1	54.9 ± 5.6*	51.3 ± 3.9*	37.1 ± 4.1
5	37.2 ± 3.5	63.0 ± 1.9	52.1 ± 5.5*	49.3 ± 4.0*	37.8 ± 3.6
15	20.7 ± 4.8*	66.3 ± 5.7	54.9 ± 5.4*	64.9 ± 4.3	36.5 ± 3.0
30	24.8 ± 3.0*	68.8 ± 4.5	49.8 ± 6.6*	61.5 ± 2.0*	37.9 ± 2.2
60	19.1 ± 1.6*	62.8 ± 5.5	46.7 ± 4.4*	55.5 ± 1.7*	29.7 ± 5.5

* $P < .05$ compared with control.**TABLE 2.** Effect of 5 s Microwave Irradiation on ENN in Cells of Donors of Different Ages (Cells monitored 5 min after irradiation)

Donor	Age (yr)	ENN (%)		
		Age norm	Control	Experimental
F	19	71.0 ± 3.0	84.6 ± 2.5	79.8 ± 3.7
G	24	72.5 ± 3.0	85.3 ± 2.7	55.0 ± 5.8*
H	26	70.0 ± 2.9	71.8 ± 2.7	51.8 ± 5.3*
I	26	70.0 ± 2.9	74.2 ± 1.5	50.1 ± 2.4*
J	26	70.0 ± 2.9	85.0 ± 1.4	74.0 ± 2.8*
K	35	64.2 ± 3.9	72.1 ± 2.0	51.2 ± 3.5*
L	38	55.9 ± 3.9	62.2 ± 2.8	53.7 ± 3.5*
M	42	48.9 ± 3.6	89.6 ± 3.4	64.0 ± 5.3*
N	52	36.0 ± 3.8	60.8 ± 2.6	47.8 ± 3.4*

* $P < .01$ compared with control.

D; and 15 s of exposure induced an increase in NEH in nuclei of donor A. Further prolongation of exposure above 15 s was not accompanied by a further increase in NEH in cells of donor B, but induced NEH increase in cells of donor D. In the cell nuclei of donor E, the initial level of NEH was nearly 100%, and therefore the influence of electromagnetic irradiation on this parameter was hardly detectable. Nevertheless, exposure for 15–60 s induced a statistically significant increase in NEH.

Taking into account the action of microwave irradiation on cell membrane functioning, the effects of heterochromatin stainability increase we observed may be connected with the increase of entrance of stain into the cell and the cell nucleus due to microwave-induced damages in cell membranes. We studied changes in cell membrane permeability by the method earlier proposed by us [Shckorbatov et al., 1995]. We did not observe any effect of microwave irradiation on cell membrane permeability to molecules of indigo carmine. Therefore, we assume that our experiments demonstrated a real increase in chromatin condensation.

TABLE 3. Electronegativity of Cell Nuclei (ENN, %) Monitored at Different Times After 5 s of Microwave Irradiation (Cells of Donor E)

Period after treatment (min)	ENN (%)
Control	77.8 ± 2.5
5	69.1 ± 2.3*
15	69.4 ± 4.2
25	71.7 ± 3.5
35	69.9 ± 1.6*
45	69.2 ± 2.0*
55	65.3 ± 2.6*
180	60.5 ± 4.4*

* $P < .05$ compared with control.

DISCUSSION

We assume that one possible mechanism for changing the electrokinetic properties of cell nuclei under the influence of electromagnetic irradiation in the millimeter range may be a change in the charged nucleus surface. The possibility of the influence of electromagnetic irradiation on the electric charge of the surface of cell membranes was shown in some theoretical work [Bond and Wyeth, 1985, Neumann, 1987], as well as in experiments [Liburdy et al., 1988, Somosy et al., 1991]. The mechanisms proposed to explain the influence of electromagnetic field in cells (detachment of charged molecules, changes of ion concentration in the double electric layer) may be applicable to our object (nuclei). We stress, however, that the nuclei dimensions of human buccal epithelium cells (approximately 10 μm in diameter) are close to the dimensions of cells investigated in other works [Liburdy, et al., 1988, Somosy, et al., 1991].

In our opinion, the other possible explanation of the influence of electromagnetic irradiation on the electrokinetic properties of cell nuclei lies in the changes of electric properties of supramolecular complexes of

TABLE 4. Effect of Microwave Irradiation on Percent of Cells Containing Nuclei With Granules of Near-Envelope Heterochromatin (NEH, %; cells stained 5 min after irradiation)

Exposure (s)	Donor				
	A	B	C	D	E
0 (control)	78.1 ± 1.7	76.8 ± 1.8	75.1 ± 2.0	78.0 ± 1.6	97.7 ± 0.5
1	83.0 ± 2.9	85.4 ± 1.4*	77.8 ± 1.4	84.8 ± 2.3	99.0 ± 0.3
5	79.6 ± 2.1	88.6 ± 1.8*	77.9 ± 1.6	91.3 ± 1.1*	98.6 ± 0.4
15	84.9 ± 2.0*	83.5 ± 9.6*	81.2 ± 1.8	96.8 ± 0.6*	99.2 ± 0.3*
30	86.4 ± 1.6*	81.9 ± 0.9*	84.4 ± 0.7*	90.1 ± 0.9*	99.7 ± 0.2*
60	84.0 ± 1.5*	84.9 ± 1.2*	85.2 ± 1.3*	90.4 ± 1.3*	99.9 ± 0.1*

* $P < .05$ compared with control.

chromatin. The aggregation of nucleoprotein complexes depends on the neutralization of their negative electric charge [Li et al., 1978]. The negative electric charge of chromatin is determined by the negatively charged phosphate groups of nucleic acids. For instance, 28% of phosphate groups of DNA in nucleohistone from calf thymus are free [Kurashina et al., 1970]. The microwave-induced decrease of ENN may reflect, in our opinion, the decrease in electric charge of chromatin and may be connected with the chromatin condensation. In our experiments, electromagnetic irradiation induced an increase in NEH and consequently chromatin condensation in cells of all donors tested (Table 4).

Our results are in good agreement with electron microscope studies that demonstrated margination and clumping of chromatin-forming electron-dense clumps of the chromatin near the nuclear membrane under the influence of different injurious chemical agents [Simard, 1970].

We suppose that changes in NEH under the influence of electromagnetic irradiation could be connected to changes in electric interactions between chromatin components that could result in decrease of ENN. However, in cells of donors B and E, an increase in NEH was not accompanied by an ENN decrease. Between NEH and ENN, in our experiments, we observed only a small negative correlation ($r = -0.346$, $P = .06$). These facts indicate that the changes in electrokinetic properties of nuclei under electromagnetic irradiation could result from different processes and that chromatin condensation is not strictly connected to changes in ENN. ENN reaction to microwave irradiation depends on the individual peculiarities of donors. These peculiarities in our relatively small group of donors did not give us the opportunity to obtain any significant correlation between ENN and age of donors.

The decrease in ENN under the influence of microwave irradiation is connected, in our opinion, with the negative action of microwaves on the cell nucleus

and on the cell as a whole. The decrease in ENN was observed under the influence on the cells of different injurious factors (high and low extremal temperatures, inhibitors of protein and RNA biosynthesis, oxidative phosphorylation, and other harmful factors) [Shckorbatov and Shakhbazov, 1992]. The fact that a microwave-induced decrease in ENN is expressed to a different extent or is absent could be regarded as an expression of the higher stability of the cells of donors with more stable ENN. The fact of different stability of ENN could be used, after further investigation, for individual selection of doses of microwave irradiation in microwave therapy, as well as in the selection of candidates for professions that have risk of exposure to microwave irradiation (work in radiolocation stations, etc.).

The fact that microwave radiation effects do not depend on time; during a 5–45 min observation after exposure may be related to a low level of metabolic activity in the cells of the upper layer of the buccal epithelium [Bhattacharya et al., 1983].

Summing up, we conclude that under the influence of microwave radiation, the electric charge of cell nuclei, estimated by means of the intracellular microelectrophoresis method, decreases in donors in which this parameter is close to or exceeds the age norm. Microwave irradiation induces an increase of the level of chromatin condensation, measured by the cytological method.

The phenomena observed have some individual specificity. We suppose that this specificity reflects important differences in the reactions of cells of different donors to electromagnetic irradiation in the millimeter range. Studies of these phenomena may reveal practical applications in medicine (via microwave therapy) and in occupational medicine.

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