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# The influence of differently polarised microwave radiation on chromatin in human cells

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**Abstract**

*Purpose:* To determine the possible biological effects of differently polarised microwave radiation on the chromatin state in human cells.

*Materials and methods:* Isolated human buccal epithelium cells were irradiated by microwaves of frequency f = 35 GHz and surface power density E = 30 $\mu$W/cm$^2$. The state of chromatin in human cells was determined by methods of light and electron microscopy. The state of cell membranes was evaluated by the method of vital indigo carmine staining.

*Results:* The microwave-induced condensation of chromatin in human cells is revealed. Degree of microwave-induced condensation depends on the state of polarisation of electromagnetic wave: In some cases left circularly polarised waves induce less effect than linearly polarised radiation. The linearly polarised electromagnetic waves induce cell membrane damage revealed by increase of cell staining. The data obtained are discussed in connection with mechanisms of biological effects of electromagnetic fields.

*Conclusion:* The data obtained in this work demonstrate important biological effects of monochromatic microwave irradiation at 35 GHz. Low-level microwave irradiation induces chromatin condensation in human cells and damages of cell membranes.

**Keywords:** *Chromatin structure, human cell, non-ionising radiation, cell biology*

## Introduction

Artificial electromagnetic fields are the important component of environment in contemporary world. The mechanisms of biological action of this factor are not clear, but some data indicate potential hazard of low-level electromagnetic irradiation.

A study of military personnel in Poland showed a significantly increased relative risk of several nervous system tumors, including brain cancer, in persons exposed to electromagnetic fields of microwave diapason (Szmigielski 1996). Odds ratios for both glioma and meningioma were slightly increased for long duration occupational exposure to microwave radiation (Berg et al. 2006). A non-significantly increased risk of brain cancer was observed among men who had ever held a job with an average magnetic field exposure $> 0.6$ $\mu$T relative to those with exposures $< 0.3$ $\mu$T. A more pronounced risk was observed among men diagnosed with *glioblastoma multiforme*. Moreover, a cumulative time index score of magnetic field exposure was significantly related to *glioblastoma multiforme* ($p = 0.02$) (Villeneuve et al. 2002).

Such investigations are numerous, but very often the epidemiological studies do not provide a clear answer to the question of whether electromagnetic field (EMF) exposure influences the development of cancer. This is due in part to two shortcomings shared by epidemiological studies: (i) Small sample number, and (ii) no specific EMF exposure parameter is known, with which correlations should be made (Hulbert et al. 1998).

Great practical interest to the problem leads to investigations of possible mutagenic effect of electromagnetic field of low intensity in radiofrequency

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diapason. Some investigations revealed mutagenic effect of radiofrequency irradiation. For instance, in the work (Garaj-Vrhovac et al. 1992) the increase of chromosome aberrations and micronuclei in human lymphocytes under microwave irradiation (frequency 7.7 GHz, power density 0.5, 10 and 30 $mW/cm^2$, cell samples exposed for 10, 30 and 60 min.) was registered. Under extended exposure conditions, radio frequency signals at an average specific absorption rate (SAR) of at least 5.0 W/kg are capable of inducing chromosomal damage in human lymphocytes indicated by micronuclei test. Exposure to each of the four signals (ranged from 837–1909.8 MHz) for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase of micronucleated lymphocytes frequency (Tice et al. 2002). The micronuclei occurrence in Chinese hamster ovary (CHO)-K1 cells exposed to microwaves of frequency 2.45 GHz at a SAR lower than 50 W/kg did not differ from the sham-exposed controls, while those at SAR of 100 and 200 W/kg were significantly higher when compared with the sham-exposed controls. An increase of SAR causes a rise in temperature and this may be connected to the increase of micronuclei formation generated by exposure to microwaves (Koyama et al. 2004).

But in other investigations no mutagenic effect of microwaves was demonstrated. For instance, irradiation by 2.45 GHz microwaves for 2 h with up to 100 W/kg SAR with continuous wave-form and an average SAR of 100 W/kg with pulse wave-form (a maximum SAR of 900 W/kg) does not induce chromosomal aberrations in m5S mouse cells (Komatsubara et al. 2005). Also there was no evidence for induction of chromosome aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 847.74 MHz radiation (Vijayalaxmi et al. 2001). Irradiation with 847.74–813.56 MHz that is applied in mobile phones at SAR 2.4–26 mW/kg does not induce alterations in level of DNA damage or induce apoptosis in Molt-4 T lymphoblastoid cells (Hook et al. 2004).

In our previous works we showed that in response to microwave irradiation in buccal cell nuclei heterochromatin granules were formed (Shckorbatov et al. 1998, 2002b) and drawn the conclusion about microwave-induced chromatin condensation in human cell nuclei. For quantitative determination of heterochromatin changes we introduced the abbreviation HGQ – heterochromatin granule quantity (Shckorbatov 1999). Cell nucleus electrokinetic properties change under the influence of microwave irradiation (Shckorbatov et al. 1998, 2001, 2002a). We also demonstrated an increase of cell membrane permeability induced by microwave irradiation (Shckorbatov et al. 2001, 2002a).

Later the fact of microwave-induced chromatin condensation was demonstrated by Markovà et al. (2005) using the method of chromatin anomalous viscosity time dependencies. A 30 min exposure to microwaves at 900 and 905 MHz resulted in statistically significant condensation of chromatin in human lymphocytes (Sarimov et al. 2004). In the work (Shckorbatov et al. 2004) we demonstrated the HGQ increase after microwave irradiation of different circular polarisation ($f = 42.25$ GGz) and also after irradiation produced by cell phone. Markovà et al. (2005) demonstrated the microwave-induced formation of foci containing tumor suppressor p53-binding protein 1. The purpose of the present work is to study the effects of low-level microwave radiation on human cells in connection with the state of circular polarisation.

## Materials and methods

### *Human cells*

Studies were performed in human cells of buccal epithelium. Cells were obtained from the inner surface of donor's cheek by light scraping with a blunt sterile spatula. This operation is absolutely bloodless and painless. All the donors were informed about the purposes of investigation. Our investigations were performed in accordance with the European Convention on Human Rights and Biomedicine (1997), Declarations and Recommendations of the First, the Second and the Third National (Ukrainian) Congresses of Bioethics (Kiev, Ukraine, 2001, 2004, 2007) and Ukrainian legislation.

The cells were placed in solution of the following composition: 3.03 mM phosphate buffer ($pH = 7.0$) with addition of 2.89 mM calcium chloride (Reachem, Moscow, Russia) and used for further experiments. A total of 25 $\mu$l of cell suspension containing several thousands of cells were placed on the glass slide and subjected to microwave irradiation. Immediately after the irradiation procedure cells were stained with orcein (Merck AG, Darmstadt, Germany) or indigo carmine (Merck AG, Darmstadt, Germany). Donors of cells were of male sex, non-smokers. Donor A was of 21 years old, donors B and C – 19 years, donor D – 35 years, and donor E – 51 years old.

### *Irradiation procedure*

As a source of electromagnetic radiation of frequency $f = 35$ GHz we applied a semi-conductor device. Irradiation was performed in a free space (10 cm from antenna edge). The experiment was conducted at a room temperature and no changes of the sample temperature during irradiation were registered. In all experiments irradiation power density at the surface of

exposed object was E = 30 $\mu$W/cm$^2$. We applied linearly polarised and circularly polarised radiation. Irradiation time in all experiments was 10 sec. The principal scheme of the experimental setup is shown in Figure 1. The SAR of the cell suspension was 0.75 W/cm$^2$. The temperature of an irradiated object in all experiments equaled the room temperature (25°C).

*Chromatin state evaluation*

In human cells we estimated the number of heterochromatin granules by the method described earlier (Shckorbatov 1999). The suspended cells (2 $\mu$l) were placed on the cover slide and irradiated. After irradiation cells were stained with 2% orcein solution in 45% acetic acid (Reachem, Moscow, Russia). Orcein is a specific stain for heterochromatin as it was shown in a classic work (Sanderson and Stewart 1961). Cells were investigated at magnification 600×. Each cell sample contained several thousands of cells. In each variant of experiment HGQ was estimated in 30 cell nuclei and the mean HGQ value and the standard error of this value were calculated (presented in Figures 4–8). This number of cells (30) was determined in our previous experiments as an optimal for such analysis. The variability of HGQ in cell population gives the value of the standard error of the mean data (SEM) less than 5% of the mean HGQ that is enough for biological experiments.

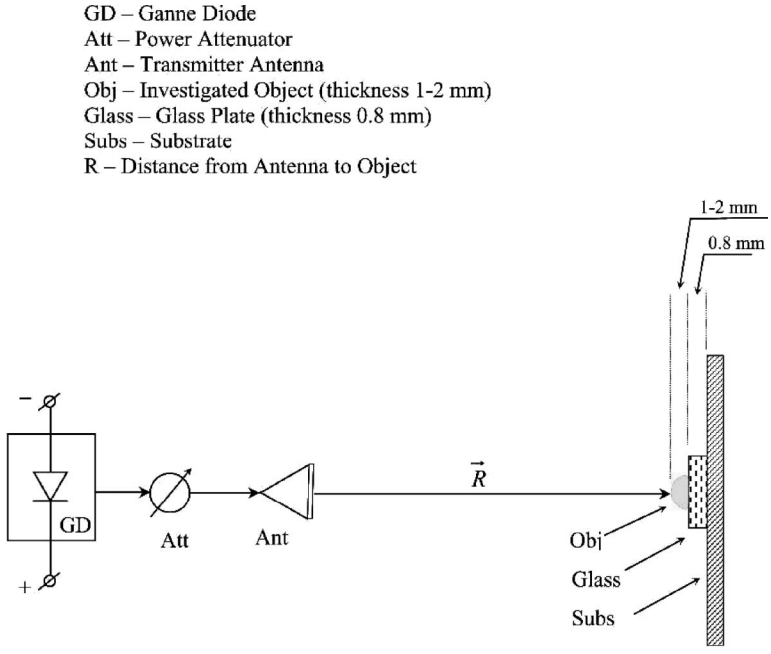


Figure 1. Scheme of the experimental set-up.

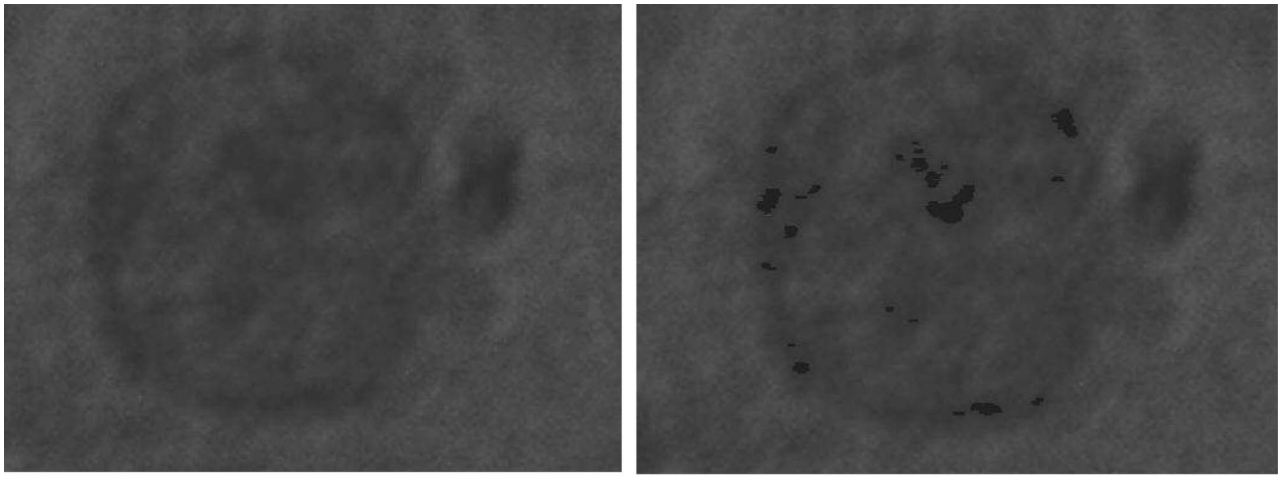


Figure 2. The nucleus of human buccal epithelium cell processed by computer program: Before (a)/after (b) the image processing (magnification 600×).

Three independent experiments for each donor were conducted using three different cell samples obtained on different days.

*Evaluation of the state of cell membrane*

We applied indigo carmine as cell damage indicator. This method also may be considered as a method reflecting cell viability. Previously it was shown that cell damage induces the increase of percentage of stained cells (Shckorbatov et al. 1995). Therefore, we used the percentage of unstained cells after 5 min of staining with 5 mM indigo carmine solution in the buffer solution described above. In one experiment we analysed three cell samples irradiated independently ($N_1$). In each cell sample we analysed 100 cells ($N_2$) and determined the percentage of unstained cells for each sample. After this we calculated the mean number of unstained cells for three experiments and the standard error of this value (presented in the Figure 10).

*Image processing*

For more distinct determination of heterochromatin location in interphase cell nucleus we developed computer program that enabled us to process the digital images and to paint zones with different heterochromatin contents in different colors. Figure 2 presents the results of image processing in colorless variant. The developed methods of computer visualisation made the work of microscopic analysis much easier and gave us the opportunity to avoid the mistakes resultant from personal subjectivity.

*Electron microscopy*

The electron microscopy investigations were done on a microscope EM-125 ('Electron' factory, Sumy, Ukraine).

*Statistical analysis*

All experimental results were statistically processed using Student's *t*-test. The probability level assumed in this paper is $p < 0.05$. Statistically significant changes compared to control are marked in Figures 5–10 with asterisks (*).

## Results

Electron microscopic image of the nucleus of buccal epithelium is presented in Figure 3. After microwave irradiation the chromatin condensation located mainly near nuclear envelope is observed (Figure 4).

The microwave irradiation of human cells induces the significant increase of HGQ parameter. As one can see in Figures 5–9, cell exposure during 10 sec

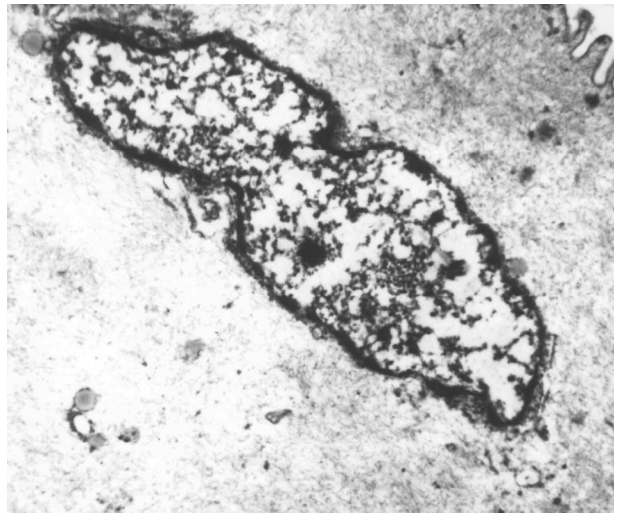

Figure 3. The nucleus of buccal epithelium cell at magnification 12,000×.

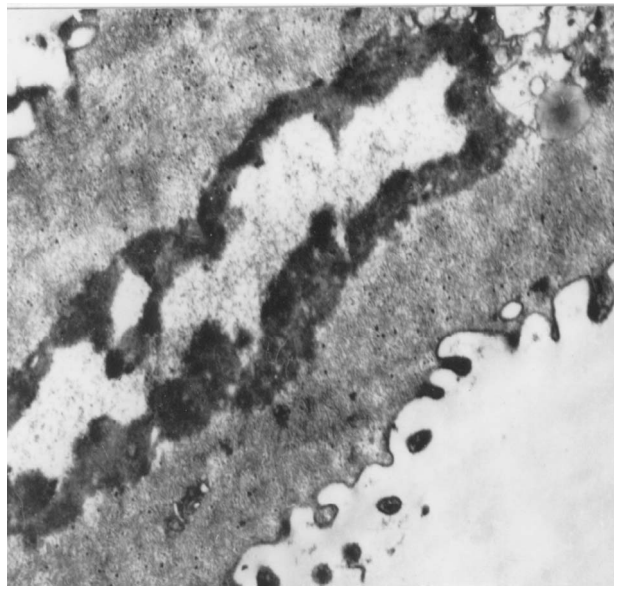

Figure 4. The nucleus of buccal epithelium cell after microwave irradiation (power density 200 $\mu$W/cm$^2$, irradiation time 60 sec, magnification 12,000×).

induces increase of HGQ. This increase was registered in all tested donors and had no relation to initial HGQ level. In cells of older donor E the initial level of HGQ was higher than in cells of other donors that is in a good agreement with our previous results indicating age-related condensation of chromatin (Shckorbatov 2001).

As one can see from the presented data, almost in all experiments the right- and left-polarised microwaves induced approximately equal biological effects, but in some cases the left-polarised electromagnetic waves induced weaker effect than right polarised one (Figure 5). In some cases linearly polarised irradiation appears to have a weaker effect than a circularly polarised one (Figure 8). The applied intensity of irradiation induces cell damage that is manifested by

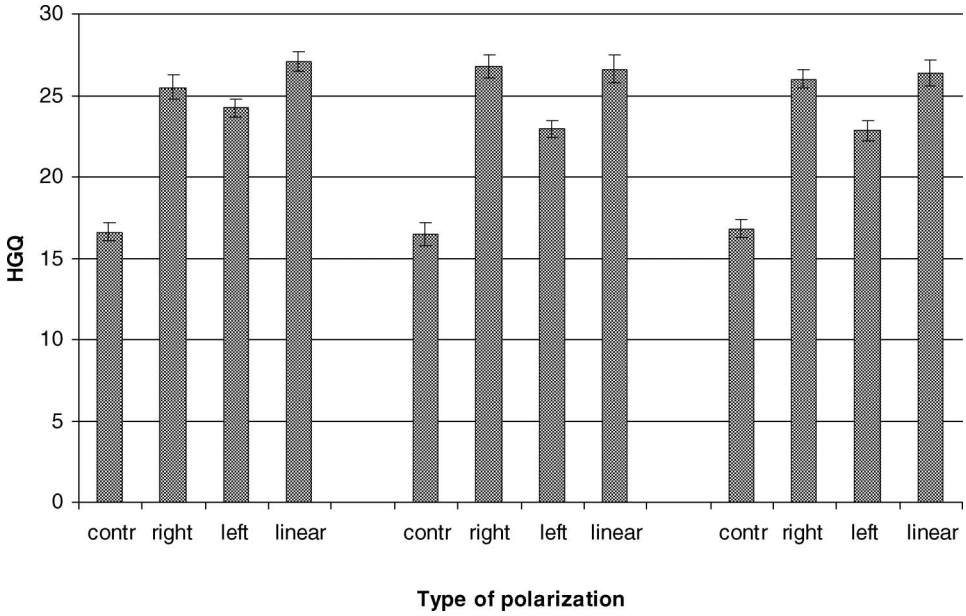


Figure 5. Changes in heterochromatin granule quantity (HGQ) after cell exposure to differently polarised microwaves in cells of donor A. Error bars indicate the standard error of the mean (SEM) for $n = 30$ independent experiments.

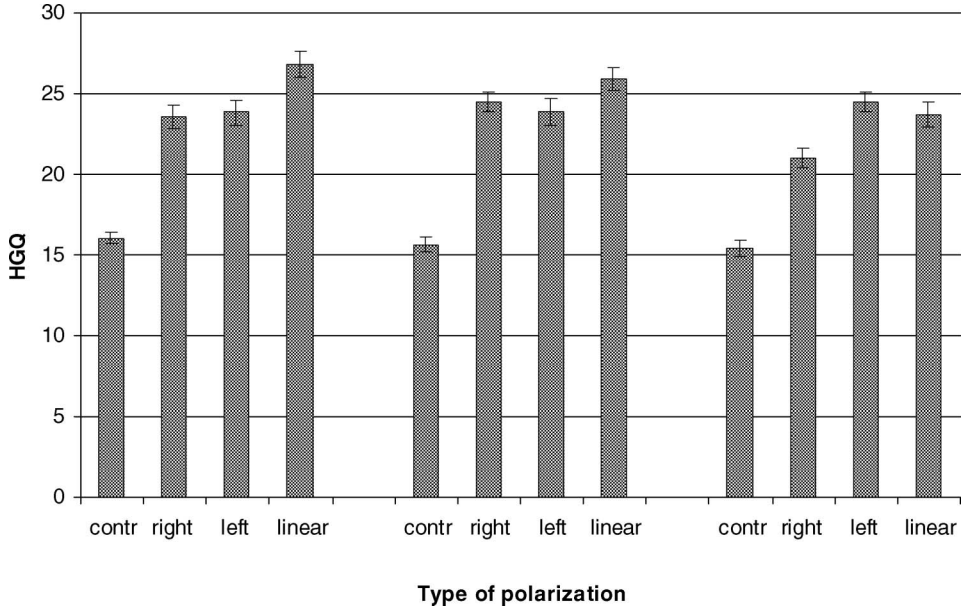


Figure 6. Changes in heterochromatin granule quantity (HGQ) after cell exposure to differently polarised microwaves in cells of donor B. Error bars indicate the standard error of the mean (SEM) for $n = 30$ independent experiments.

decreasing of percentage of unstained cells after cell exposure to linearly polarised microwaves (Figure 10).

## Discussion

The mechanism of biological action of microwaves is not yet known. Different experimental data indicate the important role of DNA and genome in it. Electromagnetic radiation induces heat shock factor activation (Lin et al. 1997) connected with EMF induction of field-responsive domain in the heat-shock protein 70 (HSP-70) promoter (Lin et al. 1999). These and other experimental facts were the basis for the hypothesis about general mechanism of EMF action via electromagnetic initiation of transcription at specific DNA sites (Lin et al. 2001). This hypothesis is based on the notions concerning the interaction of external electromagnetic fields immediately with DNA electrons (Blank and Goodman 2004). In our previous

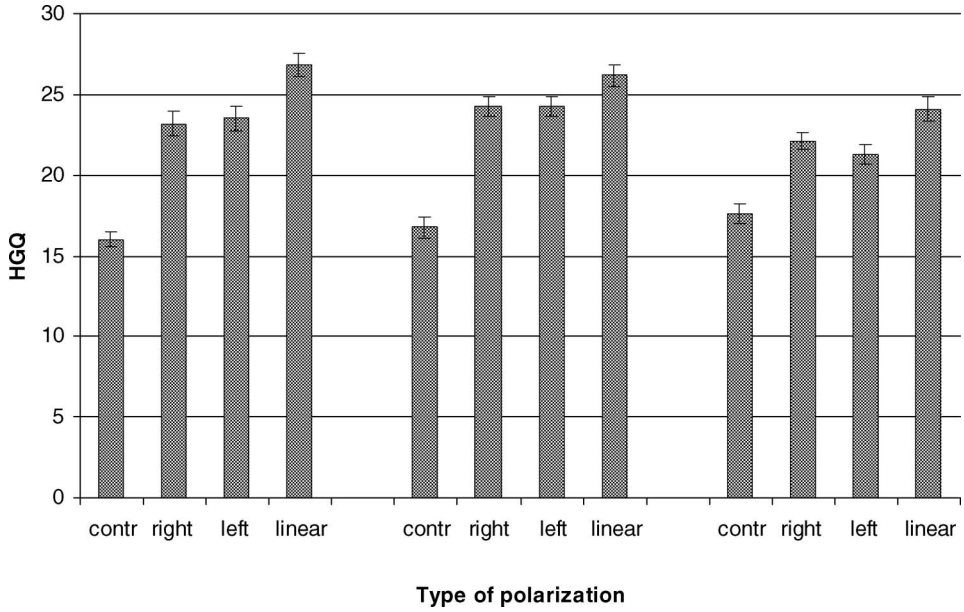


Figure 7. Changes in heterochromatin granule quantity (HGQ) after cell exposure to differently polarised microwaves in cells of donor C. Error bars indicate the standard error of the mean (SEM) for $n = 30$ independent experiments.

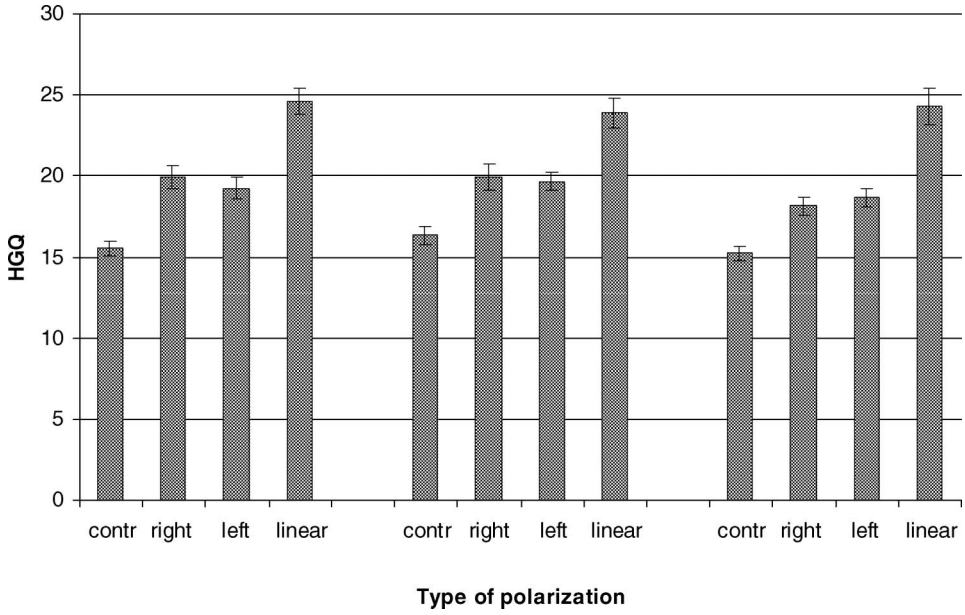


Figure 8. Changes in heterochromatin granule quantity (HGQ) after cell exposure to differently polarised microwaves in cells of donor D. Error bars indicate the standard error of the mean (SEM) for $n = 30$ independent experiments.

work we demonstrated that microwave radiation induces condensation of chromatin adjacent to the nuclear envelope (Shckorbatov et al. 1998). Our present data show chromatin condensation in the whole nucleus. We suppose that chromatin condensation is a general response to EMF irradiation. Chromatin condensation may be induced by changing of DNA-protein interaction evoked by electromagnetic filed. This possibility is proved in the work (Lin et al. 1998), in which changing of protein-DNA interaction was shown for regulatory proteins of chromatin. Chromatin condensation may in theory be a cause of mutations because it is known that chromatin condensation in chromosomes leads to mutability increase (Surrales et al. 1998).

We have demonstrated increase of number of heterochromatin granules in response to microwave irradiation. The formation of such granules in response to action of stress factors was previously demonstrated in other experimental systems. The concentration of heat shock factor 1 (HSF1) in the

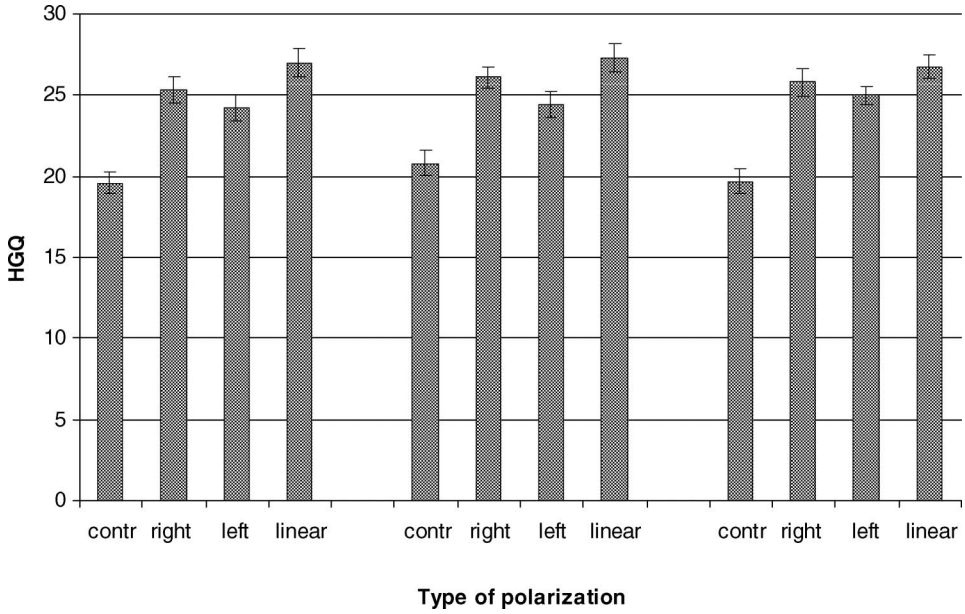


Figure 9. Changes in heterochromatin granule quantity (HGQ) after cell exposure to differently polarised microwaves in cells of donor E. Error bars indicate the standard error of the mean (SEM) for $n = 30$ independent experiments.

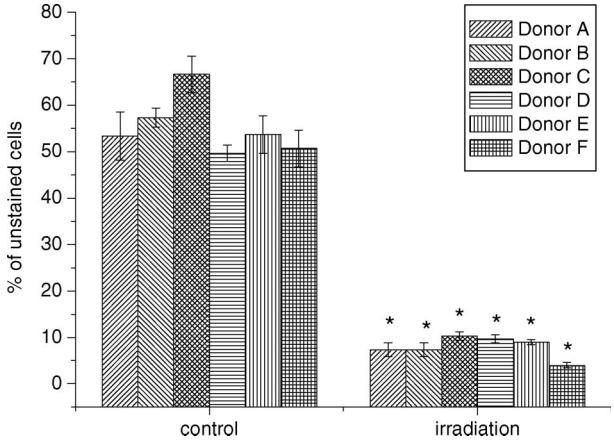


Figure 10. Changes in cell membrane permeability after cell exposure to linearly polarized microwaves. Error bars indicate the standard error of the mean (SEM) for $N_1 = 3$ independent experiments. The number of analysed cell in each experiment $N_2 = 3 \times 100 = 300$.

stress-induced interphase chromatin granules (HSF1 granules) was shown in the work (Jolly et al. 1999). HSF1 stress granules were detected within 30 sec of heat shock. HSF1 stress granules were detected after 5–10 min of treatment with cadmium, and steady state was reached after about 24 min of cadmium exposure (Jolly et al. 1999). We suppose that microwaves induce cell response to stress, which is manifested in the process of heterochromatinisation. It is known that the process of heterochromatinisation is connected with decrease of transcriptional activity (Lewin 2004). In our experiments microwaves induced cell membrane damage revealed by the decrease of the percentage of unstained by indigo carmine cells. This phenomenon supports the view that low-level microwave irradiation induces stress reaction in human cells.

Our experimental data on different sensibility of cells to differently polarised microwave irradiation may be interpreted in connection with asymmetry of biological molecules, first of all, DNA. It is known that DNA molecule is a right helix and therefore its interaction with differently circularly polarised microwaves may be the result of DNA asymmetry. Besides, such an asymmetry may lead to the differences in effect of differentially polarised EMF on heterochromatinisation.

## Conclusion

The data obtained in this work demonstrate important biological effects of monochromatic microwave irradiation at 35 GHz. Low-level microwave irradiation induces chromatin condensation in human cells and damages of cell membranes.

**Declaration of interest:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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