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THE EFFECT OF IONIZING RADIATION IN COMBINATION WITH STATIC MAGNETIC FIELD AND MICROWAVE RADIATION ON CHROMATIN STATE IN ISOLATED HUMAN BUCCAL EPITHELIUM CELLS

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The effect of gamma radiation on chromatin condensation in isolated human buccal epithelium cells was investigated. The method of staining with orcein and determination of the mean number of heterochromatin granules per one nucleus was applied, 100 nuclei were analyzed in each variant of experiment. The isolated cells of buccal epithelium demonstrate the increase of condensation of chromatin in interphase nuclei after irradiation with ^{60}Co gamma irradiation in doses 0,5–5 Gy. The effect of irradiation increases with dose up to 3 Gy, and then comes to plateau. The results of this investigation do not show enhancement of the effect of gamma irradiation in combination with magnetic field or microwave irradiation. On the contrary, we observed the pronounced decrease of the effect of gamma irradiation (2 Gy) in combination with magnetic field (25 mT) and also microwave irradiation (36,64 GHz, intensity 1 W/m^2) if it is applied before the gamma irradiation.

Keywords: buccal epithelium; cell nucleus; gamma radiation; non-ionizing radiation; heterochromatin.

The studies of cytological manifestations of the action of ionizing radiation have a long history. In this area are known classic works of Nadson and Filippov [1] and Muller [10] on genetic effects of ionizing radiation. Now new aspects of the effects of ionizing radiation on cell are studied. The most elaborately investigated are so-called genotoxic effects [11] and ionizing radiation-induced apoptosis [9] which are often studied in connection with the application of ionizing radiation in the treatment of cancer and the influence of ionizing radiation on stem cells [12]. For a long time the researchers are interested in the modifying of the effect of ionizing radiation by non-ionizing radiation – microwaves and low-frequency electromagnetic fields [2]. This problem attracts attention in connection with medical applications of ionizing radiation, but still remains unsolved.

For investigation of the biological action of ionizing radiation are applied many cytological methods. The very informative characteristic of cell state is the assessment of the degree of heterochromatinization of chromatin in cell nucleus. As it is known, the transition of chromatin from the form of the euchromatin (diffused) to the form of heterochromatin (condensed) is accompanied by a decrease in the activity of RNA synthesis [3], so the assessment of the degree of heterochromatinization can be used to determine the overall synthetic activity of chromatin. Previously it was shown that the heterochromatin granules quantity (HGQ) per one nucleus, as a characteristic reflecting the degree of heterochromatinization may be used to assess cell response to electromagnetic and magnetic fields, ultraviolet radiation, inhibitors of synthesis of RNA and protein, etc. [13]. The aim of this work was to study the effect of ionizing radiation on the degree of condensation of chromatin in interphase nucleus of isolated cells of human buccal epithelium.

Materials and Methods

Cells.

As the experimental object were used human buccal epithelium cells isolated from organism immediately before experiment. The two good-will donors of cells (men) were informed about the purposes of the investigation. The Donor A was 21 year old, Donor B – 22 years old. Cells were placed in a 3,03 mM phosphate buffer solution, pH=7,0 with addition of 2,89 mM $CaCl_2$. In this solution cells may be stored without any visible changes for several hours [12].

Source of ionizing radiation and the method of cell irradiation.

Cells suspended in the solution described above (100 μl) was placed in Eppendorf test-tube and the test-tubes were placed in a container and subjected to irradiation by ^{60}Co γ-rays at the dose rate 0,01 Gy/sec for obtaining the absorbed dose of 0,5–5 Gy. The dose obtained by the cells depended on the time of cell exposure (50 sec – 500 sec).

We also treated the cells with static magnetic field (MF) of magnetic induction 25 mT, exposure time 5 min; and microwave radiation (MW) of frequency 36,64 GHz, intensity 0,1 and 1 W/m^2, exposure time 30 seconds. The source of magnetic field was magnet, and the source of microwaves – the generator on the base of Gann diode constructed by the scientists of the Department of Theoretic Radiophysics of the Kharkiv National University. If combined treatment of cells (gamma radiation and magnetic field or microwaves) were applied, the treatment was used immediately before or after the γ –irradiation. Cells were stained with 2% orcein solution in 45% acetic acid [14] immediately after the treatment of cells with ionizing radiation or combined treatment with magnetic field or microwaves. The measure of chromatin condensation was the heterochromatin granule quantity (HGQ). It was assessed in 100 nuclei; the mean value and the standard error of the mean were calculated. The data were processed by Student's method, in Fig. 1–6 the variants that significantly ($p>0,95$) differ from control are marked with asterisks (*).

Results

The results of HGQ assessment after cell exposure to gamma rays are presented in Fig. 1–2.

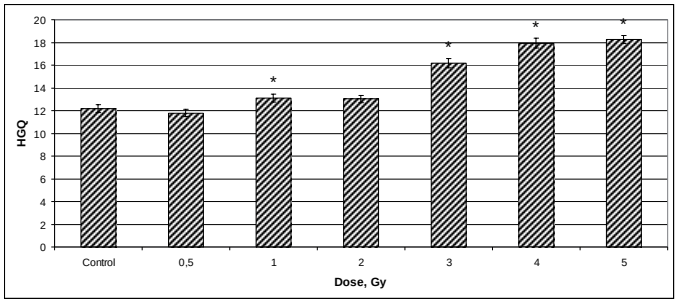

Fig. 1. The HGQ in cells of donor A after cells exposure to different doses of gamma radiation.

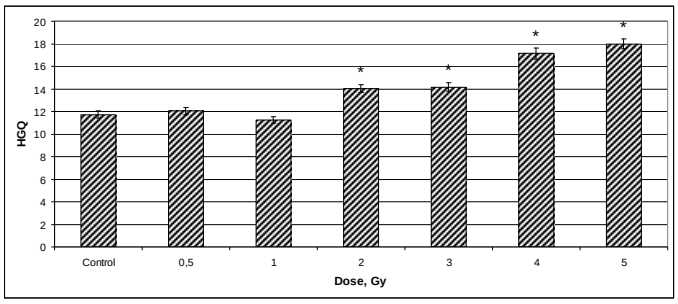

Fig. 2. The HGQ in cells of donor B after cells exposure to different doses of gamma radiation.

As one can see, even the smallest of the tested doses of gamma radiation (0,5 Gy) induces the significant increase in HGQ in cells of both donors. The further increase of the exposure dose results in the increase of HGQ. This increase is limited at the doses 4 Gy (Donor A) or 3 Gy (Donor B); the more doses induce no further HGQ increase.

In Fig. 3–4 are presented results of HGQ changes after the cell exposure to successive treatment by magnetic field (25 mT, 5 min exposure) and gamma radiation.

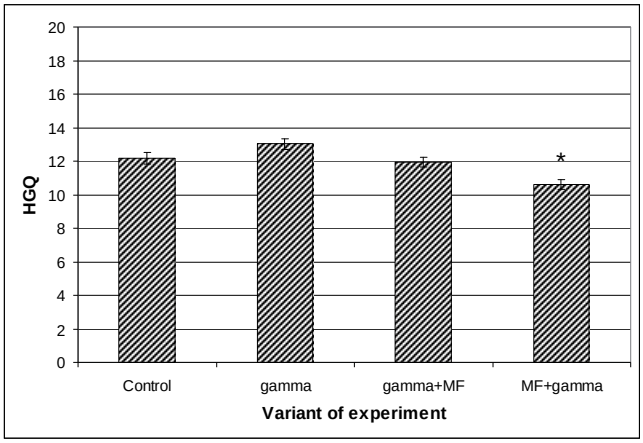

Fig. 3. The HGQ in cells of donor A after cells exposure to gamma radiation (2 Gy) and magnetic field (MF, 25 mT).

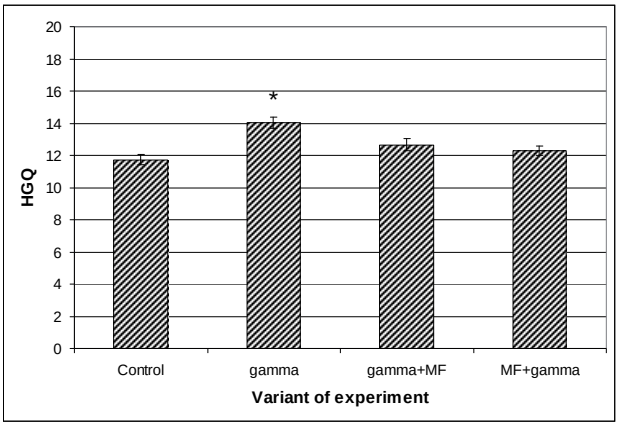

Fig. 4. The HGQ in cells of donor B after cells exposure to gamma radiation (2 Gy) and magnetic field (MF, 25 mT).

The presented data indicate that the induced by gamma radiation effect can be modified by applying of magnetic field. In the both variants: gamma radiation+magnetic field and magnetic field +gamma radiation HGQ is significantly less than in the variant - gamma irradiation.

In Fig. 5–6 are presented results on HGQ assessment after the combined irradiation by gamma radiation (2 Gy) and microwaves of two intensities: 0,1 and 1 W/m^2, exposure time 30 sec.

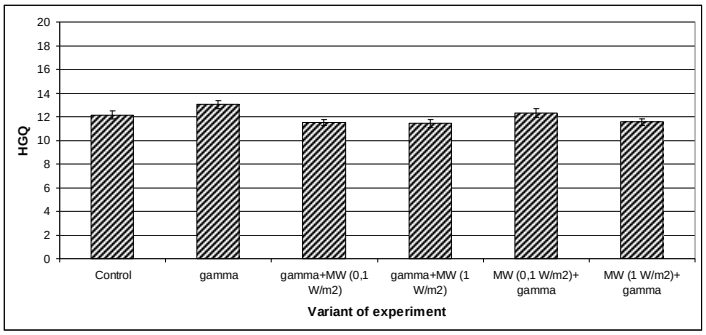

Fig. 5. The HGQ in cells of donor A after cells exposure to gamma radiation (2 Gy) and microwave radiation (MW), of two intensities, 0,1 and 1 W/m^2.

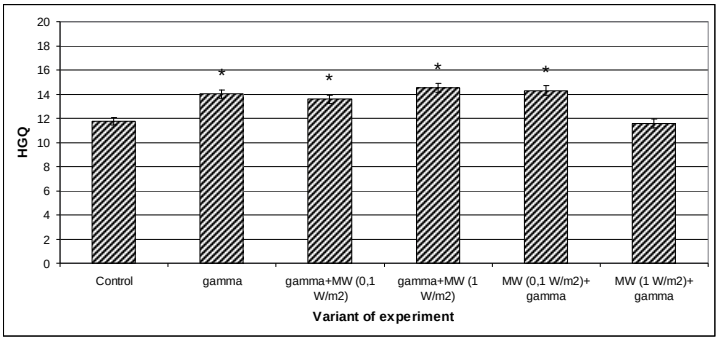

Fig. 6. The HGQ in cells of donor B after cells exposure to gamma radiation (2 Gy) and microwave radiation (MW), of two intensities, 0,1 and 1 W/m^2.

It may be seen from Fig. 5 that at the intensity 0,1 W/m^2 and 1 W/m^2 applied before gamma, the microwave irradiation significantly reduces the effect of gamma irradiation. If microwave irradiation is applied after gamma, the effect of microwaves is observed only at higher intensity 1 W/m^2. Cells of Donor B proved to be less sensitive to restoring effect of microwaves. Only in variant of combined treatment of cells: 1 W/m^2 microwaves + gamma irradiation (2 Gy) the significant decrease of the gamma radiation-induced effect of HGQ increase was observed.

Discussion

The modern data on the action of ionizing radiation on different levels of chromatin organization are summarized in [7]. According to these authors, there are several methods of determination of chromatin changes after ionizing irradiation. Hallo assay – assessment of the diameter of the fluorescent halo formed by the chromatin relaxation of the nucleus stained by the intercalating agent. Comet assay is used for assessment of DNA brakes. Pulsed Field Gel Electrophoresis technique – is used to discriminate DNA fragments according to their length. All these methods, in authors' opinion, may be used to assay effects of high doses of radiation. The method of immunofluorescence – the method of visualization of cell repair and signaling proteins, may be used for assessment of effects of low doses of ionizing radiation [7]. We are proposing not to assess the effect of chromatin decondensation (which is assessed by hallo method) occurring after applying of high doses of ionizing radiation, but the opposite process of chromatin condensation. Many stress factors induce chromatin condensation, among them the high temperature, ultraviolet radiation, microwave radiation, inhibitors of protein and RNA synthesis [13].

The data obtained show a significant increase in HGQ after the exposure to gamma irradiation (Fig. 1–2), that is a manifestation of chromatin condensation. Interestingly, that the effect of chromatin condensation is observed at doses 0,5–5 Gy in a dose-dependent manner. The HGQ comes to plateau at 3–5 Gy cell exposure. In our opinion, this is connected with limited ability of cell to react to relatively high levels of gamma irradiation in connection with increase of cell damage.

We are investigated the effects of ionizing radiation of 2 Gy combined with magnetic field and microwaves tacking in mind that the dose 2 Gy generally corresponds to a daily radiotherapy session [7]. The data obtained indicate that the effect of gamma radiation on HGQ may be reduced by applying of magnetic field (Fig. 3–4) and microwaves (Fig. 5–6) before (that is more effective in the case of microwave irradiation) and after irradiation. In our opinion, these processes may be connected with the known effect of radioadaptation [16]. The effects described in the modern literature on the combined effects of ionizing radiation and non-ionizing electromagnetic fields are quite contradictory. It s shown that extremely low frequency MF did not enhance mircronuclei

frequency induced by ionizing radiation (2 Gy), so no synergistic effects were observed [5]. Although the 1.95 GHz signal does not exacerbate the yield of aberrant cells caused by ionizing radiation, the overall burden of X-ray-induced chromosomal damage per cell in first-mitosis lymphocytes may be enhanced at 2.0 W/kg SAR [8]. But in other works is demonstrated that the damaging effect of ionizing radiation is enhanced by non-ionizing radiation. The exposure to extremely low frequency MFs immediately before or after X-ray irradiation may enhance the mutations in pTN89 plasmids [4]. The combination of electromagnetic fields (900 MHz, 2, 4, and 6 mW/cm^2, for 3 days at 2 h/d) and gamma-ray exposure (5 Gy) resulted in a synergistic effect by triggering stress response, which increased reactive oxygen species [6].

Therefore, the problem of interference of biological effects of gamma radiation and non-ionizing radiation is far from its solution. In our opinion, the cell response to the combination of these factors depends on the genotype of cells and the intensity of operating factors, and this is a cause of diversity of experimental data obtained by different authors.

Conclusion

The isolated cells of buccal epithelium demonstrate the increase of condensation of chromatin in interphase nuclei after irradiation with ^{60}Co gamma irradiation in doses 0,5–5 Gy. The effect of irradiation increases with dose up to 3 Gy, and then comes to plateau. The results of this investigation do not show enhancement of the effect of gamma irradiation in combination with magnetic field or microwave irradiation. On the contrary, we observed the pronounced decrease of the effect of gamma irradiation (2 Gy) in combination with magnetic field (25 mT) and also microwave irradiation (36,64 GHz, intensity 1 W/m^2) if it is applied before the gamma irradiation.

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ВПЛИВ ІОНІЗУЮЧОГО ВИПРОМІНЮВАННЯ В КОМБНІЦІЇ З СТАТИЧНИМ МАГНІТНИМ ПОЛЕМ І МІКРОХВИЛЬОВИМ ВИПРОМІНЮВАННЯМ НА СТАН ХРОМАТИНУ В ІЗОЛЬОВАНИХ КЛІТИНАХ БУКАЛЬНОГО ЕПІТЕЛІЮ ЛЮДИНИ

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Досліджено вплив гамма- випромінювання на конденсацію хроматину в ізольованих клітинах букального епітелію людини. Був застосований спосіб фарбування клітин орсеїном і визначення середнього числа гранул гетерохроматину у ядрі, в кожному варіанті експерименту було проаналізовано 100 ядер. Ізольовані клітини букального епітелію демонструють збільшення конденсації хроматину в інтерфазних ядрах після гамма-опромінення ^{60}Co в дозах 0,5–5 Гр. Вплив опромінення зростає з дозою до 3 Гр, а потім доходить до плато. Результати цього дослідження не показують посилення ефекту гамма-опромінення в комбінації з магнітним полем або мікрохвильовим випромінюванням. Навпаки, ми спостерігали виражене зниження впливу гамма-опромінення (2 Г) у поєднанні з магнітним полем (25 мТл), а також НВЧ опроміненням (36,64 ГГц , інтенсивність 1 Вт/м^2) при його застосуванні перед гамма-опроміненням.

Ключові слова: букальний епітелій; ядро клітини; гамма-опромінювання; неіонізуюче випромінювання; гетерохроматин.

ВЛИЯНИЕ ИОНИЗИРУЮЩЕГО ИЗЛУЧЕНИЯ В КОМБНИЦИИ СО СТАТИЧЕСКИМ МАГНИТНЫМ ПОЛЕМ И МИКРОВОЛНОВЫМ ИЗЛУЧЕНИЕМ НА СОСТОЯНИЕ ХРОМАТИНА В ИЗОЛИРОВАННЫХ КЛЕТКАХ БУККАЛЬНОГО ЭПИТЕЛИЯ ЧЕЛОВЕКА

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Исследовано влияние гамма-излучения на конденсацию хроматина в изолированных клетках буккального эпителия человека. Был применен способ окрашивания клеток орсеином и определение среднего числа гранул гетерохроматина в ядре, в каждом варианте эксперимента было проанализировано 100 ядер. Изолированные клетки буккального эпителия демонстрируют увеличение конденсации хроматина в интерфазных ядрах после гамма-облучения ^{60}Co в дозах 0,5–5 Гр. Влияние облучения возрастает с дозой до 3 Гр, а затем выходит на плато. Результаты данного исследования не показывают усиления эффекта гамма-облучения в комбинации с магнитным полем или микроволновым излучением. Напротив, мы наблюдали выраженное снижение влияния гамма-облучения (2 Гр) в сочетании с магнитным полем (25 мТл), а также КВЧ облучением (36,64 ГГц, интенсивность 1 Вт/м^2) при его применении перед гамма-облучением.

Ключевые слова: буккальный эпителий; ядро клетки; гамма-излучение; неионизирующее излучение; гетерохроматин.