ДІЯ ФІЗИЧНИХ ФАКТОРІВ НА БІОЛОГІЧНІ ОБ'ЄКТИ

UDC 576.315.4

RESPONSE OF EXFOLIATED HUMAN BUCCAL EPITHELIUM CELLS TO COMBINED GAMMA RADIATION, MICROWAVES, AND MAGNETIC FIELD EXPOSURE ESTIMATED BY CHANGES IN CHROMATIN CONDENSATION AND CELL MEMBRANE PERMEABILITY

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Modulation of the biological effects produced by ionizing radiation (IR) using microwave and magnetic fields has important theoretical and practical applications. Response of human buccal epithelium cells to different physical agents (single and combined exposure to 0.5–5 Gy γ-radiation (^{60}Co); microwaves with the frequency of 36.64 GHz and power densities of 0.1 and 1 W/m^2, and static magnetic field with the intensity of 25 mT) has been investigated. The stress response of the cells was evaluated by counting heterochromatin granules quantity (HGQ) in the cell nuclei stained with orcein. Membrane permeability was assessed by the percentage of cells stained with indigocarmine (cells with damaged membrane). The increase of heterochromatin granules quantity (HGQ), i.e. chromatin condensation was detected at the doses of 2 Gy and higher. Changes in the cell membrane permeability to indigocarmine expressed the threshold effect. Membrane permeability reached the threshold at the doses of 2–3 Gy for the cells of different donors and did not change with the increase of the dose of γ-radiation. Cells obtained from different donors revealed some individual peculiarities in their reaction to γ-radiation. The static magnetic field and microwaves applied before or after γ-radiation decreased its impact, as revealed by means of HGQ assessment.
KEYWORDS: heterochromatin, cell stress, cell damage, hormesis, static magnetic field, electromagnetic field.

РЕАКЦИЯ КЛЕТОК БУККАЛЬНОГО ЭПИТЕЛИЯ ЧЕЛОВЕКА НА КОМБИНИРОВАННОЕ ВОЗДЕЙСТВИЕ ГАММА-ИЗЛУЧЕНИЯ, МИКРОВОЛН И МАГНИТНОГО ПОЛЯ, ОПРЕДЕЛЁННАЯ ПО ИЗМЕНЕНИЮ КОНДЕНСАЦИИ ХРОМАТИНА И ПРОНИЦАЕМОСТИ КЛЕТОЧНОЙ МЕМБРАНЫ

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Проблема модификации биологических эффектов ионизирующего излучения с помощью микроволн и магнитного поля имеет важные теоретические и практические аспекты. Рассматривается реакция клеток буккального эпителия человека на действие различных физических факторов (одиночные и комбинированные воздействия γ-излучения 0,5–5 Гр (^{60}Co); микроволн частотой 36,64 ГГц с поверхностной плотностью мощности излучения на уровне объекта 0,1 и 1 Вт/м^2 и постоянного магнитного поля с индукцией 25 мТл). Стрессовую реакцию клеток определяли путём подсчёта содержания гранул гетерохроматина (СГГ) в ядрах клеток, окрашенных орсеином. Проницаемость клеточных мембран определяли по проценту клеток, окрашенных индигокармином (т.е., имеющих повреждённую мембрану). Выявлено повышение степени конденсации хроматина по показателю СГГ при воздействии дозы γ-излучения 2 Гр и выше. Изменения в проницаемости мембран имеют чётко выраженный порог: проницаемость возрастает при дозе 2–3 Гр, однако при дальнейшем увеличении дозы рост проницаемости останавливается. Клетки, полученные от разных доноров, имели индивидуальные особенности в реакции на действие γ-излучения. Применение постоянного магнитного поля и микроволн перед или после обработки γ-излучением привело к снижению стрессовой реакции клеток по показателю СГГ.
КЛЮЧЕВЫЕСЛОВА: гетерохроматин, клеточный стресс, клеточные повреждения, гормезис, постоянное магнитное поле, электромагнитное поле.

РЕАКЦІЯ КЛІТИН БУКАЛЬНОГО ЕПТІЕЛІЮ ЛЮДИНИ НА КОМБІНОВАНИЙ ВПЛИВ ГАММА-ВИПРОМІНЮВАННЯ, МІКРОХВИЛЬ ТА МАГНІТНОГО ПОЛЯ, ВИЗНАЧЕНА ЗА ЗМІНОЮ КОНДЕНСАЦІЇ ХРОМАТИНУ ТА ПРОНИКНОСТІ КЛІТИННОЇ МЕМБРАНИ

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Проблема модифікації біологічних ефектів іонізуючого випромінювання за допомогою мікрохвиль і магнітного поля має важливі теоретичні і практичні аспекти. Розглядається реакція клітин букального епітелію людини на дію різних фізичних факторів (одиночні і комбіновані впливу γ-випромінювання 0,5–5 Гр (^{60}Co); мікрохвиль частотою 36,64 ГГц з поверхневою щільністю потужності випромінювання на рівні об'єкта 0,1 і 1 Вт/м^2 і постійного магнітного поля з індукцією 25 мТл). Стресову реакцію клітин визначали шляхом підрахунку вмісту гранул гетерохроматину (ВГГ) в ядрах клітин, забарвлених орсеїном. Проникність клітинних мембран визначали за відсотком клітин, забарвлених індигокарміном (тобто, мають пошкоджену мембрану). Виявлено підвищення ступеня конденсації хроматину за показником ВГГ при впливі дози γ-випромінювання 2 Гр і вище. Зміни в проникності мембран мають чітко виражений поріг: проникність зростає при дозі 2–3 Гр, однак при подальшому збільшенні дози зростання проникності зупиняється. Клітини, отримані від різних донорів, мали індивідуальні особливості реакції на дію γ-випромінювання. Застосування постійного магнітного поля і мікрохвиль перед або після обробки γ-випромінюванням призвело до зниження стресової реакції клітин за показником ВГГ.

КЛЮЧОВІ СЛОВА: гетерохроматин, клітинний стрес, клітинні пошкодження, гормезис, постійне магнітне поле, електромагнітне поле.

At present time a special attention is mostly given to the description of biological effects of low and medium doses of ionizing radiation (IR). These doses are defined at <0.5 Gy (low) and 0.5–5 Gy (medium) [1, 2]. There are reviews dedicated to the mechanisms of ionizing radiation-induced damage [1, 3–5] and possible hormetic effect or low doses [6]. Furthermore, the problem of modification of cell effects of γ-radiation by non-ionizing electromagnetic field attracts the attention of biologists and physicians in connection to the broad usage of treatment of cancer patients with ionizing radiation (about 50% of cancer patients receive ionizing radiation treatment) [7]. The possibility of enhancement of cancer cells damages produced by ionizing radiation by the combined ELF-EMF exposure was demonstrated [8] as well as the same ability of only magnetic field regarding the effects caused by ionizing radiation [9]. On the contrary, some of investigators report about damage-reducing effects of microwaves combined with γ-radiation [10, 11].

The non-thermal biological effects of any ionizing radiation and such effects in non-ionizing range can appear only at relatively low doses (or power densities). Due to a presence both of thermal and non-thermal effects of non-ionizing radiation there is a question about ability of even low doses to affect the cell structures. It is proved that radiofrequency range radiation can cause DNA strand breaks [12]. Also there are reports about epidemiological hazard of microwaves [13] for their indirect role in carcinogenesis and even the equality of long-term GSM-range irradiation and exposure to alpha-particles in their biological effectiveness [14]. However, there is a data about the absence of microwaves exposure effects on the reproductive organs [15] and about the increase of the cell growth rate to microwaves exposure [16].

One of the most easy-to-detect cell stress response character is the state of chromatin in cell nuclei. Having two functional states (euchromatin with high transcriptional activity and heterochromatin with low one) chromatin changes its conformation under exposure to different stress factors (heat shock, chemical agents, radiation etc.) [17, 18].

Changes in cell membranes are also important to evaluate the cell damage after exposure to stress factors. Increase of cell membrane permeability under exposure to electromagnetic field was shown theoretically [19] and experimentally [20, 21]. The problem of modification

of the biological effects produced by ionizing radiation (IR) by means of microwave and magnetic field has important theoretical and practical aspects. Early research data in this field are summarized in [22].

The aim of the present work was to investigate changes in cell membranes and chromatin in response to combined exposure by ionizing, non-ionizing radiation and magnetic field.

MATERIALS AND METHODS

Human buccal epithelium cells were chosen as an experimental object because of their large size (approximately 50 μm in diameter), having a large nucleus (about 10 μm in diameter), easy for microscopic analysis, and bloodless and painless procedure of obtaining. The cells were exfoliated by spatula from inner side of cheek of 2 goodwill men donors: 19 years old donor A, and 23 years old donor B. All of them were informed about purposes and course of experiment. During the experiments cells were stored in 3.03 mM phosphate buffer solution, pH=7.0 with addition of 2.89 mM $CaCl_2$ at a temperature of 20°C [23]. After 15 minutes of exfoliation the cells were suspended in described solution and then were exposed to γ-radiation and combinations of static magnetic field or microwaves with γ-rays. Magnetic field and microwaves exposure were applied directly before or after exposure to γ-radiation.

As the source of γ-radiation was used the setup using ^{60}Co emanation "Issledovatel-1" with power of absorbed dose 0.007 Gy/sec. The dose was estimated by ferrosulphate dosimetry method [24]. Cell samples (each of 100 μl in 200 μl Eppendorf test-tube) were exposed to 0.5 – 5 Gy doses (72 – 720 sec exposure time). The source of static magnetic field with 25mT magnetic induction was a magnet. Cell suspension in Eppendorf test-tube was placed on the north pole side of magnet and treated during 5 min. Microwaves were emitted by Gann diode-based setup, constructed by Professor V.N. Bykov at the Department of Theoretic Radiophysics of the V.N. Karazin Kharkiv National University. The authors express their gratitude to Professor V.N. Bykov. The frequency of microwaves produced by the setup was 36.64 GHz, power densities were 0.1 and 1 W/m^2 (accordingly to 0.15 and 0.55 m from the surface of object to the edge of the horn antenna). A thin layer (<1 mm) of cell suspension placed on a glass slide was exposed to both power values for 30 sec. Combined exposures were conducted by using the magnetic field and microwaves before and after γ-irradiation with 15 min interval between exposures. These exposure combinations proved their efficiency in modification of γ-radiation biological effects in human cells [25].

The cell samples were examined using the MIKMED-6 var.7 microscope (LOMO, Russia) at magnification of 400x. Heterochromatin granule quantity (HGQ) was counted for assessment of the chromatin state in cell nuclei. The 2% orcein (E. Merck, Germany) solution in 45% acetic acid [27] was used for staining of cell immediately after their exposure. HGQ mean values of every variant of exposure were assessed in 3 repeats of 100 cells. In Figures are presented mean values of HGQ and SEM. Permeability of cell membrane was evaluated by staining with 5 mM indigocarmine (Sigma-Aldrich, USA dissolved in described buffer solution. Percent of stained (i.e. damaged) cells was counted in 10 repeats of 100 cells for each variant. The mean values of stainability and SEM are presented.

All data obtained were processed by Student method with Bonferroni correction for multiple comparisons. The correlation analysis was performed by Spearman method.

RESULTS AND DISCUSSION

HGQ in control not differed in cells of Donors A and B. The data presented in Fig.1 shows the slight increase in HGQ in cells of donor B after exposure to relatively small doses of γ-radiation (0.5 Gy). For cells of donor A 1 Gy dose induced statistically significant

increase in HGQ. For cells of both donors the value of HGQ at 2–5 Gy was similar, forming so-called "plateau" which is described earlier [25]. The increase in HGQ in cells of both donors was about 25% (Fig. 1).

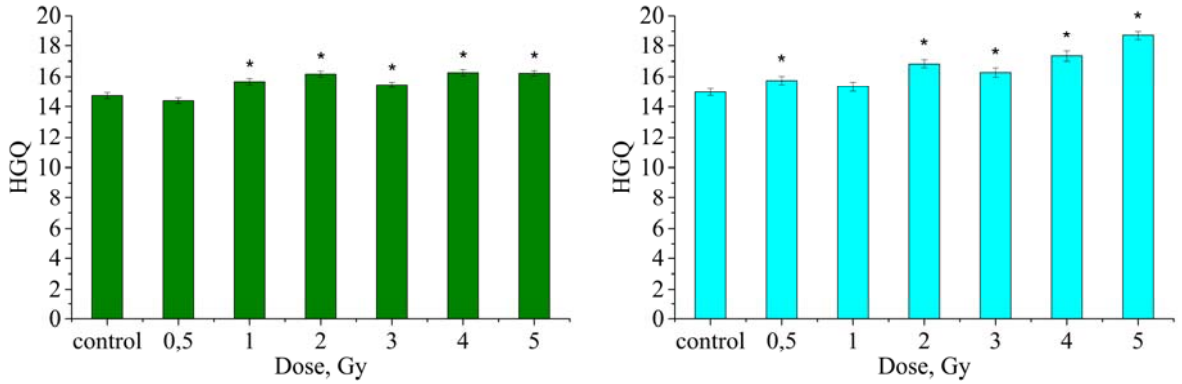

Fig. 1. Changes of chromatin state in human buccal epithelium cells nuclei after exposure to γ-radiation; a) – cells of Donor A, b) – cells of Donor; here and below the variants of experiment with significant difference ($p<0.05$) from control are marked with "*".

According to the experimental data the cells obtained from donors have variations in HGQ sensitivity to γ-radiation. The cells of Donor A revealed more sensitivity and answered by HGQ increase after the 1 Gy exposure. The cells of Donor B answered to a higher dose of IR – only to the 2 Gy exposure. Thus, the investigations of combined exposures were conducted with using the dose of 2 Gy because the significant changes of heterochromatin state at this dose were detected in cells of both donors.

Static magnetic field exposure (MF) combined with the γ-radiation exposure has led to decrease of HGQ in cell nuclei in case of pre-exposure with magnetic field (Fig. 2). Such response can be interpreted as lowering of the stress reaction caused by γ-radiation, because the increase of HGQ is connected with effects of different external stress factors [27].

These data are in a good agreement with the data demonstrating the decrease of harmful effects of IR after exposure to MF [26, 27] and contradict with results demonstrating synergetic effects of 60 Hz EMF with γ-radiation [28, 29].

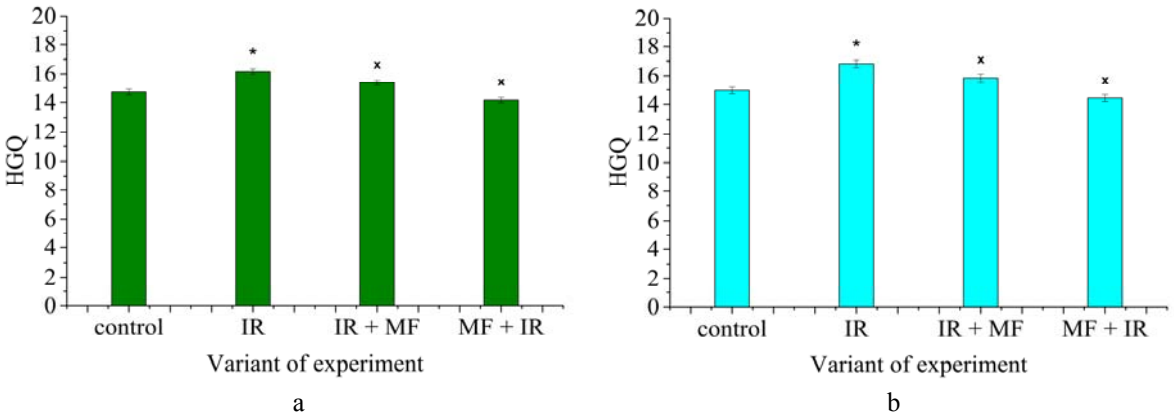

Fig. 2. Changes of chromatin state in human buccal epithelium cells nuclei after combined exposure to 2 Gy dose of γ-radiation (IR) and static magnetic field of 25 mT intensity (MF); a) – cells of Donor A, b) – cells of Donor B; here and below the difference of combined exposure variants from only γ-radiation exposure are marked with "x".

The combined exposure to microwave and γ-radiation has also led to decrease in HGQ with both power densities (0.1 and 1 W/m^2) regardless the order of exposure (Fig. 3). In cells of Donor A exposure to 1 W/m^2 microwaves before γ-radiation significantly lowered the HGQ even below the control. In cells of Donor B pre-exposure and post-exposure to both 0.1 and 1 W/m^2 microwaves of intensities reduced γ-radiation-induced HGQ elevation (Fig. 3b).

Our data are in good agreement with works [22, 30] but contradict to data of other researchers not detected synergetic or reparative effects of microwaves on DNA damage produced by γ-radiation [31, 32].

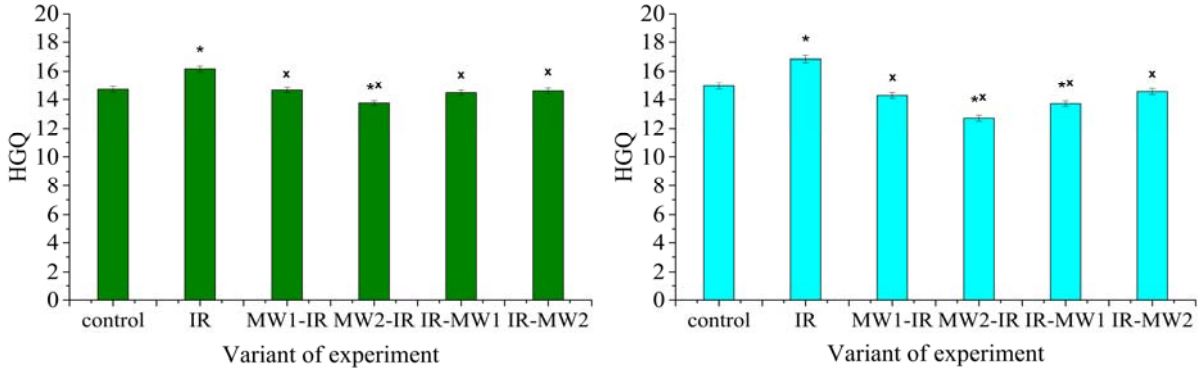

Fig. 3. Changes of chromatin state in human buccal epithelium cells nuclei after combined exposure to microwaves and gamma radiation (MW 0.1– exposure to microwave radiation of intensity 0.1 W/m^2; MW 1 – exposure to microwave radiation of intensity 1 W/m^2; and IR – exposure to gamma radiation in dose 2 Gy); a) – cells of Donor A, b) – cells of Donor B.

We demonstrated IR-induced increase of membrane permeability (increase of cell membrane permeability to indigocarmine) in cells of both donors. Changes in cell membrane permeability of donor A cells were significant only at dose of 3 Gy and above which is the evidence of reparation processes in cell membranes exposed up to 3 Gy (Fig. 4a). Changes in cell membrane permeability of donor B cells were detected at all applied doses from 0.5 to 5 Gy (Fig. 4b). The cell answer to γ-radiation by increase of membrane permeability had a threshold character. The correlation coefficient between changes in HGQ and membrane damage in cells of Donor B was 0.9.

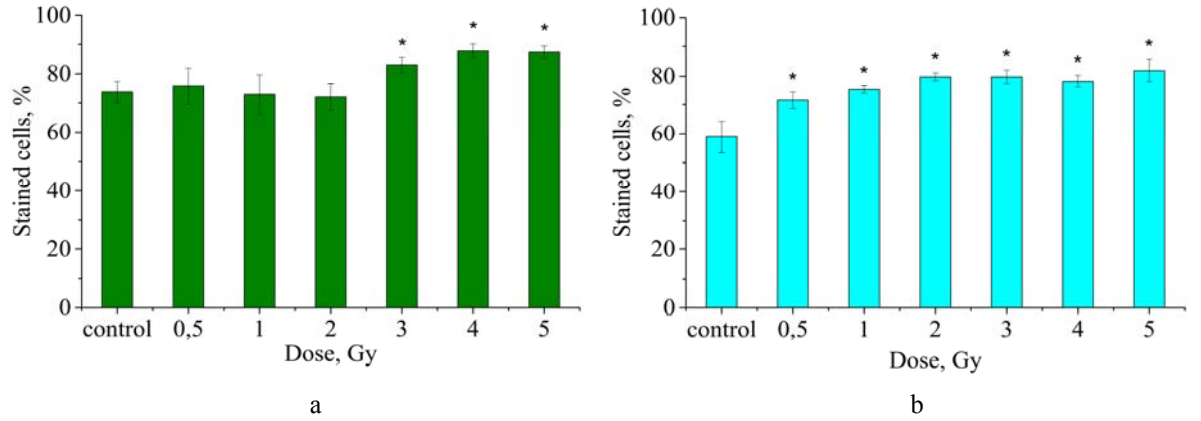

Fig. 4. Membrane permeability of human buccal epithelium cells after exposure to γ-radiation; a) – cells of Donor A, b) – cells of Donor B.

In spite of attenuating IR-induced effects detected in the HGQ, the combined exposure of γ-radiation and magnetic field led to increase of cells membrane damage (Fig. 5). The same picture was mostly observed in combination of γ-radiation with microwaves (Fig. 6). The only exception – decrease of IR-induced membrane permeability in cells of donor B by microwaves of intensity 1 W/m^2 applied before or after γ-radiation.

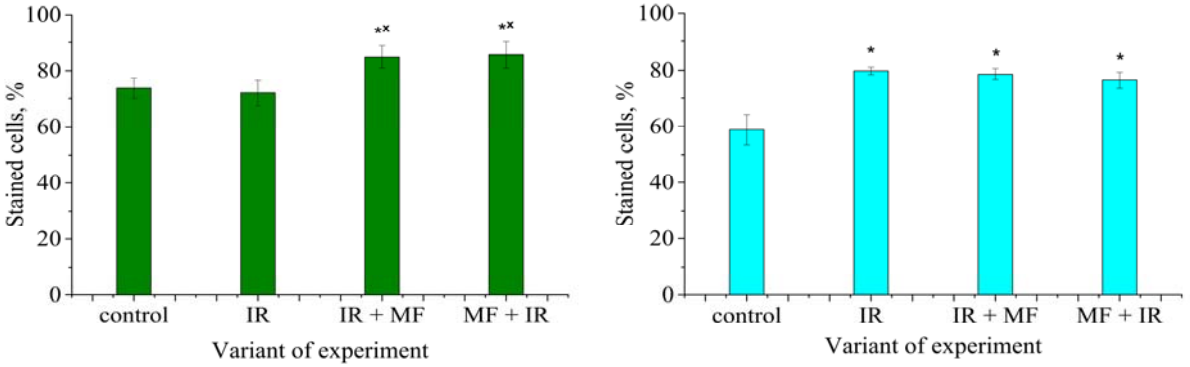

Fig. 5. Membrane permeability of human buccal epithelium cells after combined exposure to 2 Gy dose of γ-radiation (IR) and static magnetic field (MF); a) – cells of Donor A, b) – cells of Donor B.

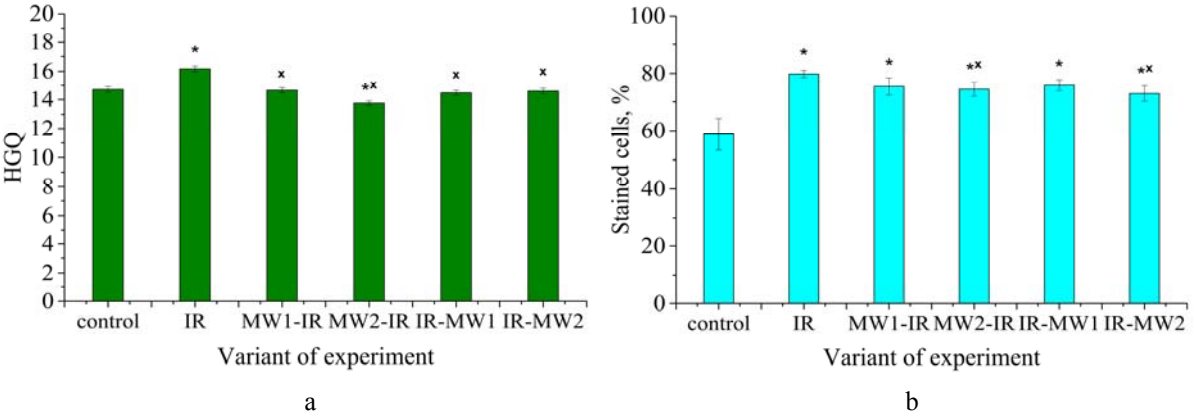

Fig. 6. Membrane permeability of human buccal epithelium cells after combined exposure to microwaves (MW – 0.1 W/m^2 and 1 W/m^2) and to γ-radiation of 2 Gy dose (IR); a) – cells of Donor A, b) – cells of Donor B.

Our experimental data demonstrate the distinct modifying effect of magnetic field/ microwaves on γ-radiation induced cell response measured by chromatin condensation. This effect is in good agreement with cell responses to electromagnetic and magnetic field applied in combination with ionizing radiation [22, 26, 27, 30–32]. In our opinion, this is connected with the leading role of cell nucleus in cell resistance to stress factors.

CONCLUSIONS

The response of buccal epithelium cells to medium doses of ionizing radiation (0.5 – 5 Gy) results in changes of chromatin state. The increase of heterochromatin granules quantity (HGQ), i.e. chromatin condensation was detected at 2 Gy dose. Cell answer to γ-radiation revealed some donor-dependent peculiarities in sensitivity to applied factors. The cell response with the increase of dose to sub-lethal values (4 – 5 Gy) revealed the slowing-down or even absence of further HGQ increase after 2 Gy dose. Changes in cell membranes permeability to vital dye expressed the threshold effect. Membrane permeability reached threshold at the dose 2 – 3 Gy for cells of different donors. The γ-radiation-induced

heterochromatinization was less expressed under exposure both to static magnetic field or microwaves combined with γ-radiation exposure. In most cases the γ-radiation-induced cell membrane damage became even more expressed after combined exposure with magnetic field or microwaves. Only in cells of one donor the microwave irradiation (1 W/m^2) revealed protective effect on cell membranes. Thus, the effect of reversion the γ-radiation-induced effect on chromatin condensation by magnetic field and microwaves is shown. γ-radiation-induced increase of cell membranes permeability not demonstrate such distinct effects in the same experimental conditions.

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