



(RESEARCH ARTICLE)



## Cell stress response to low-dose neutron radiation

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### Abstract

**Background.** It is a point of discussion whether low-dose ionizing radiation has harmful or stimulating impact on cell. According to high relative biological effectiveness of neutron radiation there is a need of description of any process triggered in the cell by neutrons.

**Objective.** The aim of current work is the investigation of the low dosed neutron radiation effects on human cells by indicators of cell stress such as state of chromatin and cell membrane permeability.

**Materials and methods.** Human buccal epithelium cells from 3 male donors (21, 24, 25 years old) were exposed to fast neutron radiation in dose range 2.3–146.0 mSv from <sup>239</sup>Pu-Be source. State of chromatin was evaluated by count of heterochromatin granules quantity in 100 nuclei stained with 2% orcein in 45% acetic acid; ratio of cells with increased membrane permeability stained with 5 mM indigocarmine in 300 cells.

**Results.** Changes in level of heterochromatin granules quantity and in cell membrane permeability revealed wave-shaped dependency with maximum effects at 36.5 mSv. Further increase of dose resulted in return of both chromatin state and membrane permeability levels closely to control or even lower.

**Conclusion.** Membrane restoration and chromatin decompaction under doses higher than 36.5 mSv together can be a sign of hormetic (stimulating) effect of low-dose neutron radiation.

**Keywords:** Cell Nucleus; Chromatin; Membrane Permeability; Adaptation; Hormesis

### 1. Introduction

The study of the of neutron radiation influence on biological objects is of great interest both when used in medicine and to study the mechanisms of the biological effect of corpuscular ionizing radiation on different types of tissues and to establish the link between biological response and the functional (proliferative) activity of cells.

The process of cell stress development in response depends on both type and intensity of the radiation. In general the relative biological effectiveness (RBE) of neutron radiation is counted as about 2–20 times higher than the one for X- or  $\gamma$ -rays according to kind and energy range of particles [1], [2]. However there is the data that states about different values of neutron radiation RBE, in some separate cases this index may reach 21 [3] or even 26 [4]. Lesser absorbed doses of neutron radiation inflict greater damage to the cells, which is manifested in the inhibition of gene expression,

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whereas X-rays may have both suppressive and enhancing effects [5]. But at the same time there is information about weak influence of neutron radiation on patients' survivability [6].

The effects of neutron radiation at the range of high doses are far more investigated and show non-linear but still clear "dose-effect" dependence, for example among rat [7] and human cells [8]. But even for now there are a lot of questions regarding the biological response to low dosed neutron radiation. Such concepts as "bystander effect" (non-targeted influence) [3] and "Petkau effect" (increased significance of low dose effects comparing to higher doses) [9] are discussed. Since one there have been the results about the increase of carcinogenesis risks [10] and frequency of chromosomal aberrations [4] in the range of low doses (i.g. neutrons), there has been also the information of the insignificance of such effects [11]. Moreover, the data about positive influence of low doses of ionizing radiation on animal organisms exists [12].

In this regard, studies of the effect of neutron radiation effects on human cells are perspective. A successful and promising model for such studies is human buccal epithelium cells. These cells can be obtained in their native form during a bloodless and painless collection procedure; they are convenient for assessing cell stress by various indicators, such as chromatin state and cell membrane permeability.

The aim of present article is to investigate human buccal cells nuclei and membrane reaction to the exposure to low dose of neutron radiation.

## 2. Material and methods

### 2.1. Cell culture

Human buccal epithelium cells in this investigation were obtained from 3 male non-smoking donors 21 (A), 24 (B) and 25 (C) year old. Small quantity of donors in our research is used due to necessity of investigation of response to neutron radiation exposure at cellular level firstly and of individual changes possibility at cell membrane and chromatin condition secondly.

Cells were scraped from cheek mucosa with a sterile blunt spatula. Cells were placed in the 3.03 mM phosphate buffer solution with addition of 2.89 mM CaCl<sub>2</sub> (pH=7.0).

### 2.2. Cell exposure procedure

The sources of neutron radiation were two Pu-Be sources IBN-17 (Russia) (energy of neutrons in range 100 keV – 10 MeV, with a mean energy at 4.5 MeV, total intensity –  $10^8$  n/s at  $4\pi$ , distribution is isotropic). Thermal neutrons were blocked by using 2 mm Cd shield. The intensity low energy gamma-radiation (mostly 59 keV) emitted by the neutron source was blocked by 2 mm layer of Pb [13]. Range of doses received by cells and exposure time are shown in Tab.1

**Tab 1** Equivalent doses of neutron radiation received according to the overall exposure period

Exposure time, min.	1	2	4	8	16	32	64
Equivalent dose, mSv	2.3	4.6	9.2	18.3	36.5	73.1	146.0

### 2.3. Sample preparations

All experiments were done at 23 °C. For heterochromatin granules quantity assessment cells were stained for 30 min by 2 % orcein (Merck, Germany) solution in 45 % acetic acid [14]. Cells were stained immediately with mentioned compound after exposure to radiation. Heterochromatin granule quantity (HGQ) was assessed in totally 100 nuclei.

Cell membrane permeability (CMP) for vital dyes was evaluated by staining with 5 mM indigo-carmin (Sigma-Aldrich, USA) dissolved in described buffer solution. Stain was added immediately after cell exposure for 5 min. After that, samples of cells were photographed, and the percent of stained (i.e. damaged) cells was counted in 10 repeats of minimal total value of 300 cells for each experimental variant. CMP was estimated from ratio between quantity of stained cells and total number of cells analyzed [15].

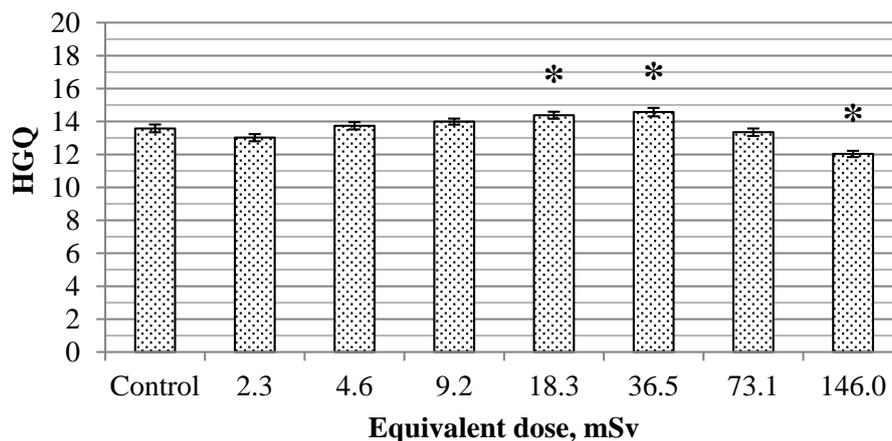
Cells were analyzed in photographs made at magnification 400x. MICMED-7 microscope (Russia) and Eyepiece Digital Microscope Camera HDCE-30C (China) were used to obtain microphotographs for analysis.

#### 2.4. Statistic processing

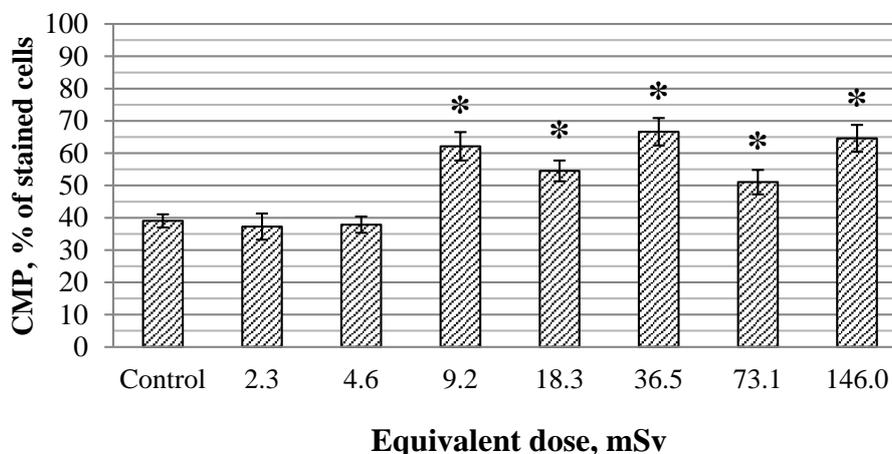
The mean values of heterochromatin granules quantity and cell membrane permeability for cell sample and standard error of the mean (SEM) were calculated, the results were processed by Student's method. In all upcoming figures the mean values and SEM are presented. Statistically significant differences ( $p < 0.05$ ) between control values and exposed ones after Student's method are marked with “\*”.

### 3. Results and Discussion

Comparison of heterochromatinization and cell membrane permeability indexes in cells of donor A shows the difference in triggering doses: 18.3 mSv for chromatin condensation and 9.2 for membrane permeability. Cells at exposure to maximal dose (146.0 mSv) revealed a contradictory process which consisted of CMP kept at almost the same value as mentioned 36.5 mSv-peak, but significant decrease of heterochromatin granule quantity (HGQ) index to even lower level – to 90% of control. Generally, chromatin decondensation process interlaced with damaged membrane. In addition, the wavy changes in cell membrane permeability (CMP) index were detected. Such dependency may reflect the reparative processes which could be held by upcoming portion of neutron radiation dose.



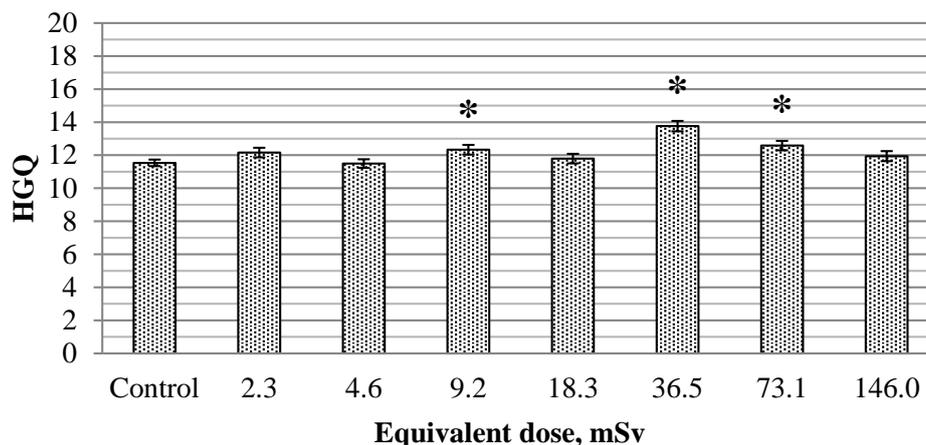
(A)



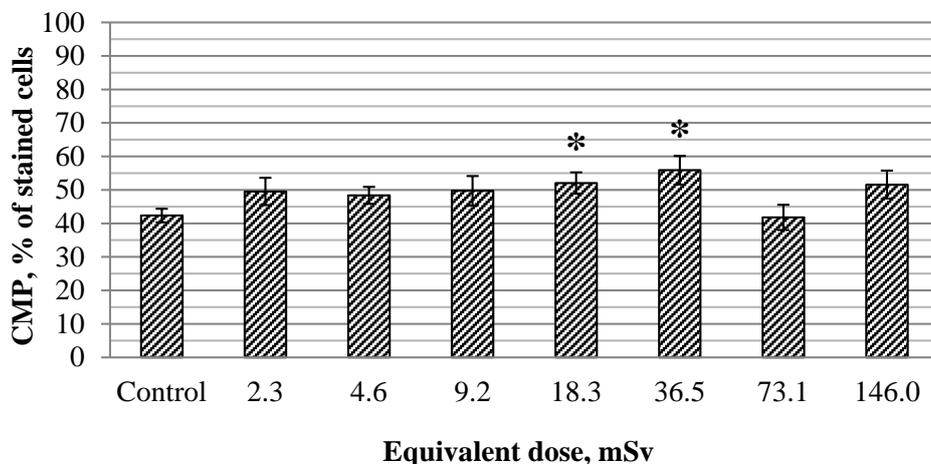
(B)

**Figure 1** Chromatin condensation (A) and cell membrane permeability (B) of donor A after exposure to low-doses of neutron radiation (“\*” is statistically significant difference from control value,  $p < 0.05$ ).

State of donor B's (Fig.2) cells included HGQ and CMP 36.5 mSv-peak but had contrariwise to donor A's trigger doses for changes in chromatin condition (9.2 mSv) and membrane permeability (18.3 mSv). Changes in chromatin with dose increase led the HGQ index straightly to control level at 146.0 mSv dose. Membrane permeability was also decreased, but no waves of CMP fluctuation were detected due to their statistical insignificance.



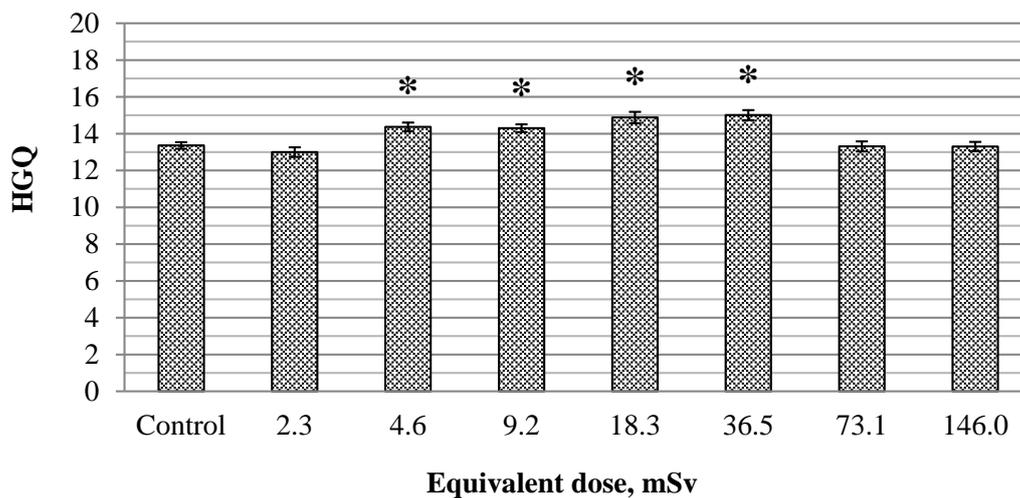
(A)



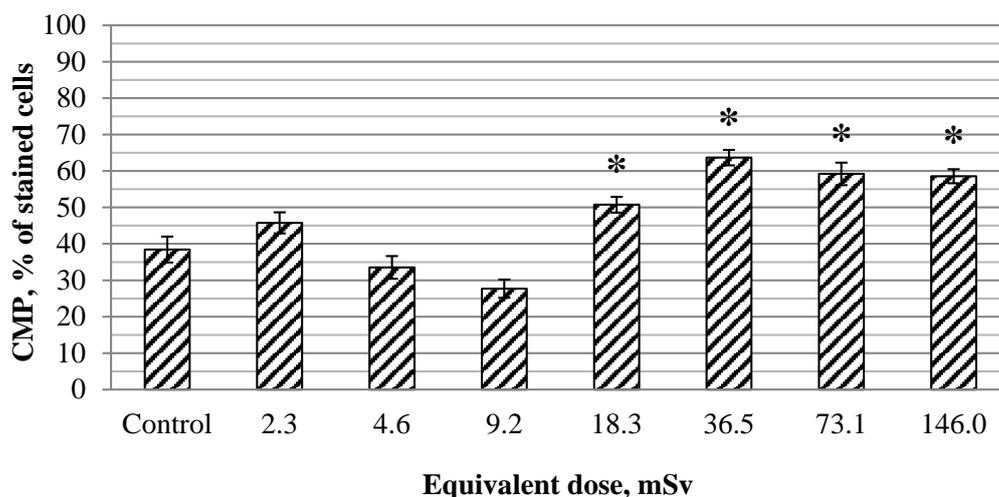
(B)

**Figure 2** Chromatin condensation (A) and cell membrane permeability (B) of donor B after exposure to low-doses of neutron radiation (“\*” is statistically significant difference from control value,  $p < 0.05$ ).

Chromatin condition in cells of donor C was the most sensitive to neutron radiation having the trigger value of 4.2 mSv, however, the changes in cell membrane appeared in the manner closer to processes in donor B's cells: the first significant changes in permeability was observed at dose of 18.3 mSv; any wavy changes were absent, CMP index had also 36.5 mSv-peak and slight decrease with higher doses (Fig. 3).



(A)



(B)

**Figure 3** Chromatin condensation (A) and cell membrane permeability (B) of donor C after exposure to low-doses of neutron radiation (“\*” is statistically significant difference from control value,  $p < 0.05$ ).

The data presented in Fig. 1 – 3 show what the low doses of neutron radiation are able to induce increase both in HGQ (chromatin condensation) and CMP (membrane permeability) indices. Cells of different donors revealed individual features in the response as well as the similarity in the peak of the chromatin condensation and membrane permeability increase at 36.5 mSv with slight further fading of the stress response to the control levels or even lower.

The investigations of biological effects of neutron radiation are mainly dedicated to the doses above 0,5 Gy (or 1 – 10 Sv of equivalent dose). Such doses are significant for antitumoral therapy, but the range of low doses is still unclear. However, there is an evidence of strong influence of neutron radiation on the rate of chromosomal aberrations linearly from the dose and non-linear link between dose and apoptosis rate [4].

By the data in [16] it is stated that the low-dose radiosensitivity below 1 Gy with radioresistance to doses above 1 Gy is observed also in human cell (e.g. salivary gland cells). Buccal epithelium cells may respond adaptively in similar way to the irradiation. On the other part, the described effects can be explained from point of view that refers to the theory of hormesis. Response of the cells to higher doses (73.1 – 146.0 mSv) went through the time period 32 – 64 min. (Tab. 1), this time was enough for reparative processes activation to deal with stress effect inflicted by lesser doses. Such case correlate with the report about the ability of the membrane to completely restore itself within 1 hour [17]. According to that the primary chromatin condensation at the range of doses 4.6 – 18.3 mSv decreased which also had a reflection

in the rate of cell membrane permeability. The restoration of membrane barrier functions is a sign of biosynthesis processes ongoing in the cell.

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#### 4. Conclusion

Very low dose neutron radiation 4.6 – 18.3 mSv in dependence of donor of cells induces chromatin condensation and increase in cell membrane permeability of human buccal epithelium cells with maximum effect at 36.5 mSv. Interestingly, the higher irradiation doses – 73.1 and 146 mSv induce no chromatin condensation or even decondensation of chromatin. Cell membrane permeability increases at 9.2 – 18.3 mSv doses of neutron irradiation also at 36.5 mSv. Higher doses of neutron irradiation – 73.1 and 146 mSv cause no increase in permeability of cell membrane or even decrease of this characteristic to level lower than in control. Cell exposure to a dose 2.3 mSv induces no changes in chromatin condensation and membrane permeability. Both chromatin decondensation and absence of increase of membrane permeability after cell exposure to doses 73.1 and 146 mSv may be explained by stimulating (hormetic) effect of neutron radiation at low-dose range. The hormetic effect is acquired in the course of irradiation – smaller doses of irradiation induce hormetic effect that is manifested then cells are exposed to higher dose.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

Authors declare no conflict of interests.

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