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CYTOGENOTOXIC EFFECTS OF EXPOSURE TO IONIZING RADIATION IN THE MINERS BUCCAL CELLS

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Abstract
The study results of the cytogenetic damages in the buccal cells of miners working in conditions of the natural ionizing radiation exposure are presented. It is shown that genotoxic effects in the buccal cells of the miners are manifested in the reducing of the normal cell frequency, in the increase of the necrotic cells and in the increase of the binuclear cells.

Introduction
Underground mining of ores containing of the natural radionuclides such as uranium, thorium, radium is dangerous to the health of workers due to mixed irradiation of the different types of ionizing radiation.

The micronucleus test is the most widely used to evaluate the genotoxic effects of ionizing radiation sources. It is used in the cultivation of peripheral blood cells in the cytokinetyc block (Пелевина и др., 2011). However, in certain cases, such effects are preferable to study on buccal epithelium allowing to obtain information about the genetic changes in human cells with minimal invasive procedures (Holland et al., 2008). This method was first used in 1983 (Stich et al., 1983) and have been used when monitoring of the environment genotoxicity.

The aim of our study was to determine the genotoxic effects of the natural ionizing radiation sources formed during underground mining on the health of miners by using micronucleus test on buccal epithelial cells.

Materials and Methods
Loparite ore, along with precious metals (Ti, Ta, Nb, etc.), contains impurities of natural radionuclides (^{238}U, ^{232}Th, ^{226}Ra), which are a source of mixed types of ionizing radiation. Buccal cells are the first barrier (Holland et al., 2008) arising in the way of carcinogens entering the body by breathing, with water and food.

The consent of the donors was obtained. The donors were questioned about they age, time of exposure, habits such as smoking, drinking, and consumption of drugs. The all donors were male, smokers and they were age varieties between 25 and 40 with addiction to smoking. The exposed group consisted have been included of 10 miners who had been working on the loparite ore minning (Revda, Murmanskaya oblast'). The unexposed group consisted of 8 individuals (Apatity, Murmanskya oblast'). They were selected because they had socioeconomic characteristics similar to those of the exposed group but they were not in direct contact with environment or occupational radioactive agents.

The subjects were required to rinse their mouths with water before sampling. Buccal cells sampling and their preparation according to Meyer et al. (Мейер и др., 2010). Microscopic analysis was performed using light microscope AXIOSTAR PLUS (Karl Zeiss, 15x40).

One slide was prepared for each subject and 1000 cells were evaluated per slide to determine micronucleus frequencies and nuclear changes. Micronucleus and other nuclear abnormalities were classified according to Tolbert et al. (1992). Cells with micronucleus were identified by the presence of both a main nucleus and one or more smaller nuclear structures. A micronucleus should be less than one-third the diameter of the main nucleus; on the same plane of focus; have a same colors, texture and refraction as the main nucleus; have a smooth, oval or round shape and be clearly separated from the main nucleus (Tolbert et al., 1992). Besides micronucleus, other nuclear abnormalies such as karyolysis, pyknosis, karyorrehxis, vacuolizated nucleus, fragmented nucleus, apoptosis, binucleus, nuclear bud and nuclear cut were recorded separately.

Registration of the mixed types of ionizing radiation (alpha, beta, gamma and neutron radiation) was performed in the mines using certified procedures on certified hardware by the employees of the regional laboratory of radiating control (RLRC), Institute of Chemistry Technology of Rare Elements and Mineral Raw Materials, Kola Science Centre the Russian Academy of Sciences.

Results and Discussion

Measurement of natural radionuclides (^{232}Th и ^{238}U, ^{40}K) on the ore body surface and in different compartments in the Lovozero district mine shaft (Revda) have shown that exposure dose at the ore body exit surface was 1.9 mSv/h while the gamma-ray background and the exposure rate throughout the mine were in the range of 0.5-1.5 mSv/h in the absence of tehnogenic radionuclides (^{137}Cs, ^{90}Sr et al). The daughter products of ^{226}Ra, ^{228}Ra, ^{224}Ra such as radon, thoron, RaA, RaV and RaS were found in the airspace in of all mine sections. The maximum concentrations of radon (20,000 Bq/m3) were found at the working face and in poorly ventilated areas (Петрашова и др., 2011). Thus, the miners were exposed to chronic irradiation by mixed sources of ionizing radiation of natural origin.

The majority of buccal cells have a large cytoplasm relative to the nucleus and the shape of the cell is angular rather than spherical, the exception is basal cells. Genomic instability or genotoxic insult in the basal cells leads to chromosome breakage or loss and micronucleus formation. Some cells with genome damage may be eliminated via the apoptotic process. The daughter cells from the basal layer differentiate into "prickle cells" which are eventually differentiated into the flattened and keratinized surface mucosal cells which exfoliate from the surface of the oral lining. Each of these cell types may contain micronucleus (Holland et al., 2008). Photographies of the different buccal epithelial cell types are presented (Fig. 1).

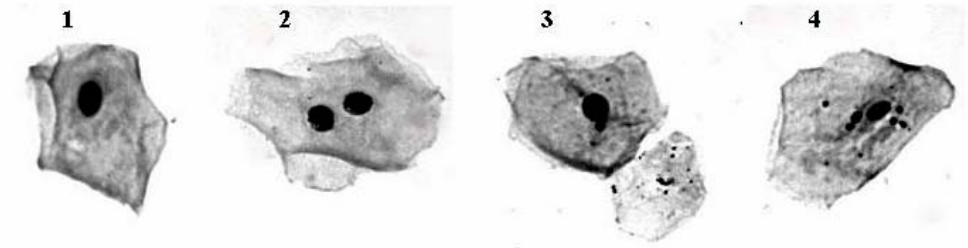

Fig. 1. Different buccal epithelial cell types: 1 – with normal nucleus, 2 - binucleate cell; 3 – cell with micronucleus; 4 – cells with apoptosis.

The cytogenetic characteristic frequencies in the exposed and unexposed groups are presented in the table. You can see that the frequency of cells with a normal nucleus in 2 times more in the unexposed group than in the exposed group (p = 0.0002). Miners have in 1.8 times more cells with karyolysis (p = 0.0064), in 12.4 times more cells with karyorrhexis (p = 0.0107) and in 2.26 times more binucleate cells.

Table. Cytogenetic characteristic frequency (%)

Type	M±m		Significance level
	unexposed group	exposed group	
Cells with normal nucleus	**58.70±5.09**	**29.23±3.86**	**p=0.0002**
Cells with micronucleus	0.16±0.04	0.11±0.038	p=0.3675
Cells with karyolysis	**27.02±3.84**	**49.25±5.53**	**p=0.0064**
Cells with pyknosis	4.26±1.08	6.81±1.09	p=0.1202
Cells with karyorrehxis	**0.38±0.18**	**4.72±1.44**	**p=0.0107**
Cells with vacuolizated nucleus	3.54±0.67	6.14±1.68	p=0.2076
Cells with fragmented nucleus	4.03±0.55	2.13±0.87	p=0.1025
Cells with apoptosis	1.88±0.37	0.93±0.29	p=0.0561
Binucleate cells	**0.016±0.016**	**0.42±0.09**	**p=0.0015**
Cells with nuclear bud	-	0.09±0.04	
Cells with nuclear cut	-	0.17±0.07	

The significant difference was not found between exposed and unexposed groups in relation to frequencies of micronucleus (p=0.37). The frequency of cells with apoptosis were at 2 time higher in the unexposed group than the exposed group (p=0.06). When all types of cytological and nuclear disorders had been combined by the type of cell death (apoptosis or necrosis) it was found that the buccal cell death by necrosis type occurred in 1.9 times more often in the exposed group (66.9% vs 35.2%, p=0.0001). That is necrosis type of cell death was dominated. At the same time, the buccal cell death by apoptosis type occurred in 1.8 times more often in unexposed group than in the exposed group (5.9% vs 3.3%, p=0.0771).

Thus, we have shown that cytogenetic features in miner's buccal epithelium cells, induced by the natural

radiation, include the substantial decrease of the normal cells, the increase of the number of cells with necrotic changes and the increase more than an order of the binucleated cells in compare with the control group. The sharp increase of the binucleate cells in the buccal epithelium of examinees from exposed group in compare with the unexposed group could indicate the influence of the ionizing radiation on the process of cytokinesis. The studies on the cell cultures have shown that the number of multinucleate cells in cell cultures of different ontogenetic and phylogenetic origin increased synchronously with the increasing intensity of the neutron component of the Earth's surface by solar proton events (Belisheva et al., 2012). It is possible that the neutron component contribution when miners irradiating by mixed sources of ionizing radiation can be manifested in the increase of the multinucleate cells frequency in the miners' buccal epithelium. The increase of binucleate cells frequency in 2.26 times in the miners' buccal epithelium and apoptosis reducing in 1.8 times in compared with the unexposed group indicates the low efficiency of the apoptosis of the miners designed to eliminate the defective genetic material. This is an unfavorable prognostic sign and indicates a high degree genotoxicity of mixed types of ionizing radiation in mining production.

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References

Мейер А.В.. Дружинин В.Г., Ларионов А.В., Толочко В.А. Генотоксические и цитотоксические эффекты в буккальных эпителиоцитах детей, проживающих в экологически различающихся районах Кузбасса // Цитология. – 2010. – Т.52, №4. – С.305-310.

Пелевина И.И., Афанасьев Г.Г., Алещенко А.В., Антощина М.М, Готлиб В.Я., Конрадов А.А., Кудряшова О.В., Лизунова Е.Ю., Осипов А.Н., Рябченко Н.И., Серебряный А.М. Молекулярно-клеточные последствия аварии на ЧАЭС // Радиац. биология. Радиоэкология. – 2011. – Т. 51, № 1. – С. 154-161.

Петрашова Д.А., Белишева Н.К., Пелевина И.И., Мельник Н.А., Зользер Ф. Генотоксические эффекты в буккальном эпителии горняков, работающих в условиях облучения природными источниками ионизирующего излучения // Известия Самарского научного центра Российской академии наук. – 2011, Т. 13, №1(7). – С. 1792-1796.

Belisheva N.K., Lammer H., Biernat H.K., Vashenyuk V.E. The effects of cosmic rays on biological systems – an investigation during GLE events // Astrophysics and Space Sciences Transactions, 2012, 8. – P. 7-17.

Holland N., Bolognesi C., Kirsch-Volders M., Bonassi S., Zeiger E., Knasmueller S., Fenech M. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage. The HUMN project perspective on current status and knowledge gaps // Mutat Res. – 2008. – Vol.659(1-2). – P.93-108.

Stich H.F., San R.H., Rosin M.P. Adaptation of the DNA-repair and micronucleus tests to human cell suspensions and exfoliated cells // Ann. N.Y. Acad. Sci. – 1983. – Vol. 407. – P. 93–105.

Tolbert P.E., Shy C.M., Allen J.W. Micronuclei and other nuclear anomalies in buccal smears: methods development // Mut. Res. – 1992. – 271. – P. 69-77.