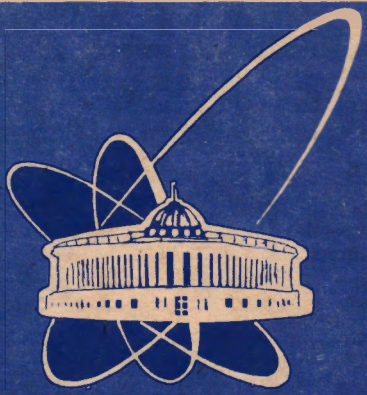

ОБЪЕДИНЕННЫЙ
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ИССЛЕДОВАНИЙ

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E.A.Krasavin, R.D.Govorun, B.S.Fedorenko[1], V.M.Petrov[1],
S.Kozubek[2], E.Lukášová[2], M.V.Repin, S.V.Druzhinin

CYTOGENETIC EFFECTS OF HEAVY IONS IN HUMAN LYMPHOCYTES

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[1]State Scientific Centre of RF — Institute for Biomedical Problems, Moscow, Russia
[2]Institute of Biophysics AS CR, Brno, Czech Republic

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INTRODUCTION

The study of radiation-induced damages in genetic structures enables to evaluate the "genetic risk" of radiation exposure and, on the other hand, the certain types of genetic damages induced by radiation reflect the level of radiation exposure and can be considered as a biological dosimeter. As known, the most utilized method to quantify human radiation exposure is the score of dicentric chromosomes in human metaphase lymphocytes. The chromosomal analysis of cosmonauts' lymphocytes reveals the increase of dicentrics and rings after long space flight. This fact undoubtedly indicates on the radiation factor as an origin of these types of aberrations and reflects the important role of galactic heavy charged particles. The number of chromosomal aberrations decreases up to spontaneous level after space mission and it can be connected with elimination of unstable chromosomal aberrations.

The level of stable chromosomal aberrations can be used as an indicator of radiation exposure. For the understanding of the nature of chromosomal lesions it is important to know the frequency of stable aberrations for radiation with different linear energy transfer (LET). Recently, techniques of molecular biology, such as fluorescence *in situ* hybridization (FISH) with whole-chromosome specific probes, made possible to detect the stable chromosomal aberrations after different doses of radiation [1]. The study of the induction of stable chromosomal aberrations in human lymphocytes induced by heavy ions was the goal of this investigation.

CHROMOSOMAL ABERRATIONS IN LYMPHOCYTES OF COSMONAUTS

The analysis of unstable chromosomal aberrations in lymphocytes of cosmonauts before and after space flights has been done. The study was per-

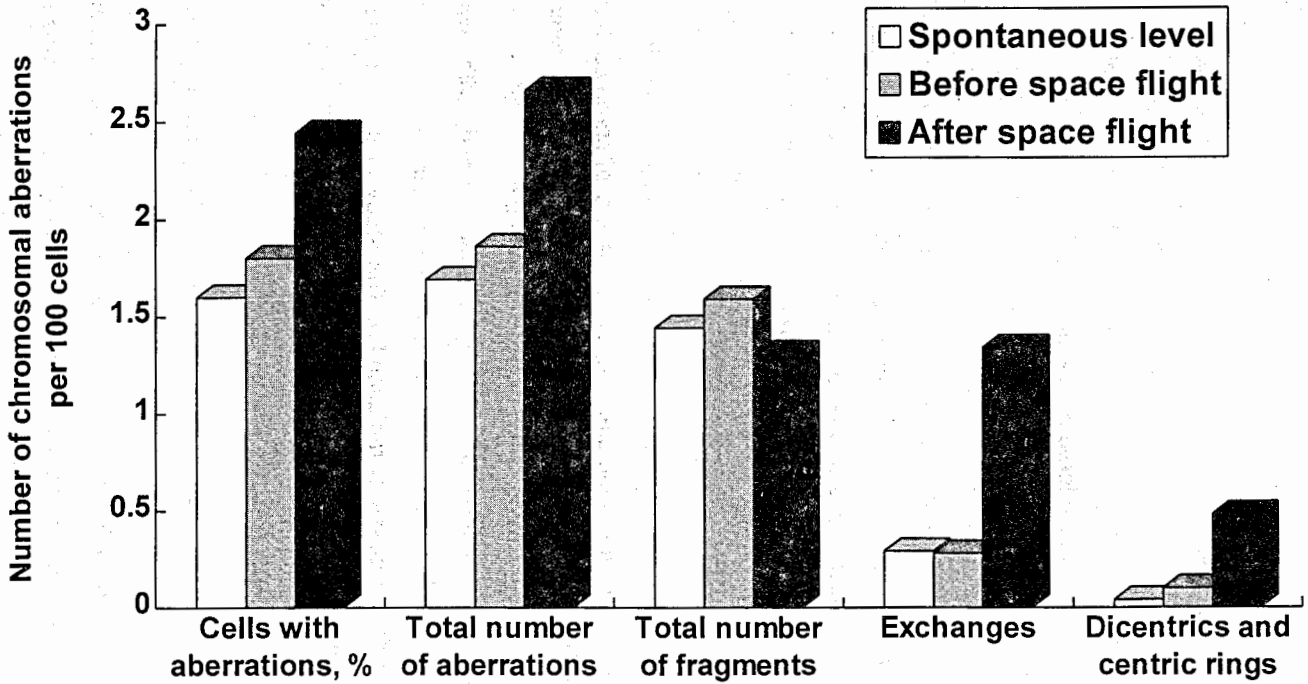

Fig. 1. Chromosomal aberrations in peripheral blood lymphocytes of cosmonauts.

formed for 23 cosmonauts. 16 cosmonauts from the group were examined before the first flight. This group was considered as a control. Air-dried preparations were made in a routine way. Slides to be scored for the presence of chromosomal aberrations were stained with aqueous Giemsa solution.

The generalized results of the frequency of chromosomal aberrations in lymphocytes of cosmonauts before the flight (1–2 months) and in a day after the flight are presented in Fig. 1. The total duration of the flights was from 117 to 515 days. As can be seen the level of cytogenetic lesions in lymphocytes before the flight did not differ from the spontaneous level. The average number of the cells with chromosomal aberrations was approximately 1.6–1.8% (the fluctuations were from 0.8% to 3.5%). The total number of the chromosomal aberrations was 1.7–1.9 per 100 cells. The number of dicentrics and rings that usually are considered as an indicator of radiation action did not exceed 0.1 per 100 cells. It is not marked the essential distinctions on frequency of deletions and exchanges of chromosomes. The frequency of chromosomal lesions in cosmonauts' lymphocytes before the flight corresponds to accepted norms for the healthy people of young age.

The analysis of chromosomal aberrations in lymphocytes of cosmonauts reveals the tendency of their increase after the flight. The number of the cells with chromosomal aberrations and their total number were increased up to 2.45% and 2.66%, respectively. The increase of the total chromosomal aberration number was determined by the frequency of exchange aberrations including dicentrics and centric rings. The frequency of chromosomal deletions did not change.

The absorbed doses of ionizing radiation for different groups of cosmonauts were in the range of 1.0–30.9 cSv and did not depend from the flight duration. The frequency of chromosomal lesions did not depend also from the absorbed dose. The following flights were not earlier than in a year after the previ-

ous one. In the period between flights the frequency of chromosomal aberrations was decreased up to spontaneous level.

These results enable to conclude that low doses of ionizing radiation during the long space flight cause the chromosomal aberration formation. This effect is not marked for the short space flights [2, 3]. The increased level of dicentrics and rings points out on the role of radiation factor in origin of these chromosomal aberrations. Taking into account that the cells with unstable chromosomal aberrations eliminate quickly with the time it is important to study the peculiarities of formation of stable chromosomal aberrations that preserve in cell population for a long time after irradiation with different LET. FISH-technique allows to carry out this analysis.

HUMAN LYMPHOCYTE CHROMOSOME-1-ABERRATIONS INDUCED BY γ–RAYS AND HEAVY IONS

Cytological preparation

Human lymphocytes were prepared from the whole heparinized blood of healthy donors. Plasma containing lymphocytes was obtained after 1.5 h of blood settling at the room temperature. In experiment with γ–rays it was divided into 3 ml fractions and irradiated by different doses of ^{137}Cs γ–ray source with the dose rate of about 3.6 Gy/min. Irradiation with nitrogen ions was performed on the accelerator of heavy ions U-400M, Joint Institute for Nuclear Research. The energy of nitrogen ions was 50 MeV/nucleon. Lymphocytes were irradiated in special plates 4 mm deep and 12 mm in diameter (Fig. 2). In this case 0.4 ml of the plasma was placed into such plates. The plates were set into a round magazine, that was managed by remote control to the beam exit window. The LET of nitrogen ions with energy 50 MeV/nucleon as a function of path length in water is shown in Fig. 3. Control and irradiated samples were diluted (1:3) with

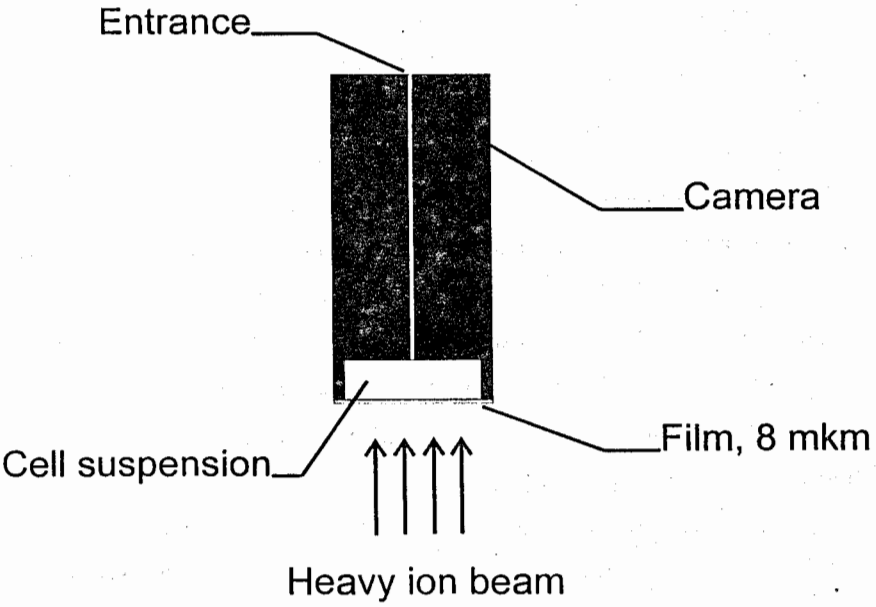

Fig. 2. Camera for irradiation of the cells by heavy ions

RPMI-1640 medium containing 0.1 ml of PHA (Murex, England) per 5 ml suspension and incubated for 51 and 57 hours after γ-ray and heavy ion radiation, respectively. Colchicine was added 2 h before the end of incubation. After hypotonic treatment the lymphocytes were fixed in methanol/acetic acid (3:1) and metaphase spreads were prepared. The slides were stored at -20° C under nitrogen atmosphere until fluorescence *in situ* hybridization.

Fluorescence in situ hybridization

Biotinylated Cotasome 1 total chromosome probe (Cambio, England) was used to stain chromosome-1 by FISH technique in spreads fixed on microscopic slides according to the method recommended by Cambio. After 16 h of hybridization at 42°C, the slides were washed in 50% formamide, 2xSSC (pH 7.0) for 15 min at 37°C followed by washes in 2xSSC with 0.1% Nonidet (Serva) for 5 min at 43°C. The slides were equilibrated in PBD for 5 min. The detection of biotinylated probe was achieved by incubation of slides with 60 μl of fluorescein-labelled extravidin (FITC-extravidin, Sigma) for 30 min at 37°C followed by three washes in 4xSSC with 0.1% Tween-20 for 5 min each at 37°C. The slides were dehydrated and mounted in Vectasheld (Vector) containing 0.3 μg/ml of propidium iodid (PI). Preparations were examined under Zeiss microscope equipped with dual filter (AHF, Germany) specific for FITC and PI. The images were scanned by cooled color videocamera with high resolution capacity (CCD camera C5310, Hamamatsu) and stored in the computer memory using the frame-grabber (MuTech, Co.).

RESULTS

The frequencies of different aberrations of chromosome-1 after irradiation by γ-rays and nitrogen ions are presented in the Table. Abberations of this chromo-

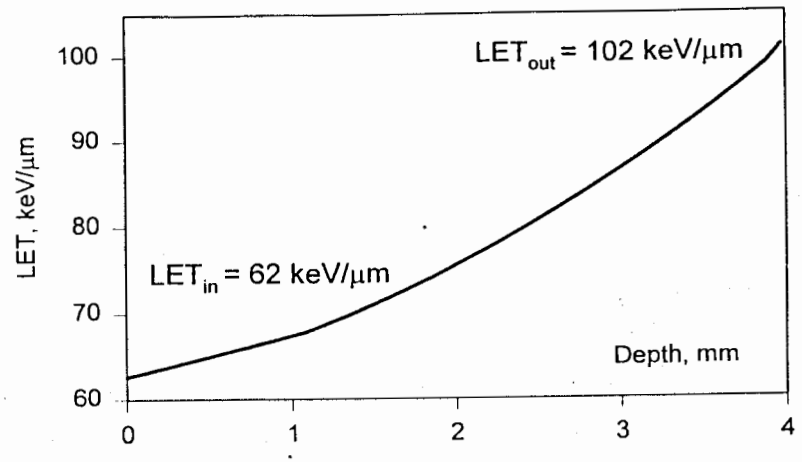

Fig. 3. LET of nitrogen ions (50 MeV/A) as a function of path lengt in water (average LET = 77 keV/μm).

$$L = \frac{\int \frac{dE}{dx} dx}{\int dx}$$

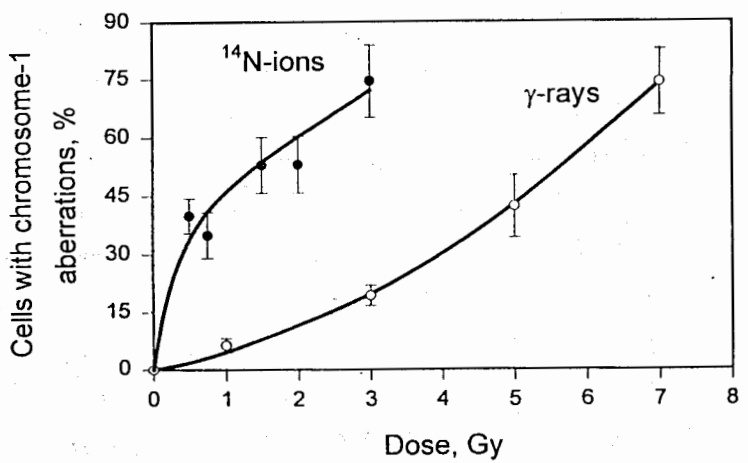

Fig 4. The frequency of cells with aberrations of chromosome-1 in human lymphocytes as a function of γ- and nitrogen ion radiation doses.

some were not observed in control non-irradiated samples. The aberrations of chromosome-1 after irradiation are very frequent. After γ–irradiation there are 6.4% of cells with different types of chromosome-1 aberrations at the dose of 1 Gy and the number of such cells increases with growing of dose and reaches 74% at 7 Gy.

After irradiation with nitrogen ions the rapid increase of the frequency of the cells with chromosome-1 aberrations (up to 35–40%) has been noted at the doses 0.5–0.75 Gy. The level of such cells reaches 74% at 3 Gy. As it follows from Fig. 4 the dose-response dependence for aberrant cells reaches the plateau at the level of 75–80%. This fact reflects, obviously, the influence of cell division delay, especially for the cells with multiple chromosomal aberrations [4, 5].

It would follow from the data of Table that the action of both types of radiation leads to the high frequency of stable chromosome-1 aberrations. As can be seen translocations prevail amongst other types of aberrations after γ-irradiation. Approximately 1/2 of all aberrations were translocations. The frequency of translocations was characterized by linear-quadratic dose dependence (Fig. 5). After heavy ion irradiation the level of translocations was more higher in comparison with γ-rays. The linear dose-responce dependence was observed. At the same time the data show the high frequency of chromosome-1 fragments induced by heavy ions (up to 40% of the total number of aberrations): they predominate after irradiation with heavy ions. So the fraction of translocations was 25% of all aberrations only.

The total number of chromosome-1 aberrations detected by FISH technique are shown in Fig. 6. The linear dependence as a function of dose was revealed after nitrogen ion irradiation. After irradiation by γ–rays the linear-quadratic dose-response dependence was found.

Table. Chromosome-1 aberrations of human lymphocytes after γ-rays and nitrogen irradiation

Dose, Gy	Cells scored	Cells with aberrations, %	Total aberrations, per 100 cells	Aberrations, per 100 cells				
				Translocations	Insertions	Dicentrics	Rings	Fragments
γ-rays								
0	150	0	0	-	-	-	-	-
1	206	6.4±1.8	6.4±1.8	2.5±1.1	1.5±0.9	0.5±0.5	0	1.9±1.0
3	279	19.4±2.6	19.4±2.6	10.5±1.9	2.5±0.95	2.0±.0.8	0	4.4±1.3
5	169	42.2±5.0	46.3±5.2	21.0±3.5	2.5±1.2	7.0±2.0	1.3±0.9	14.5±2.9
7	100	74.0±8.6	83.0±9.1	40.0±6.3	4.0±2.0	10.0±3.2	2.0±1.4	27.0±5.2
Accelerated heavy ions (^{14}N)								
0	200	0	0	-	-	-	-	-
0.5	200	40.0±4.5	52.5±5.1	7.5±1.9	0.5±0.5	2.5±1.1	15.0±2.7	27.0±3.7
0.75	100	35.0±5.9	43.0±6.6	13.0±3.6	4.0±2.0	4.0±2.0	11.0±3.3	11.0±3.3
1.5	104	52.9±7.1	73.1±8.4	19.2±4.3	1.0±1.0	10.5±3.2	12.5±3.5	29.7±5.3
2	101	53.0±7.2	90.0±9.4	22.0±4.7	1.0±1.0	9.0±3.0	22.0±4.7	36.0±6.0
3	86	74.4±9.3	153.5±13.4	40.7±6.9	10.5±3.5	5.8±2.6	11.6±3.7	86.0±10.0

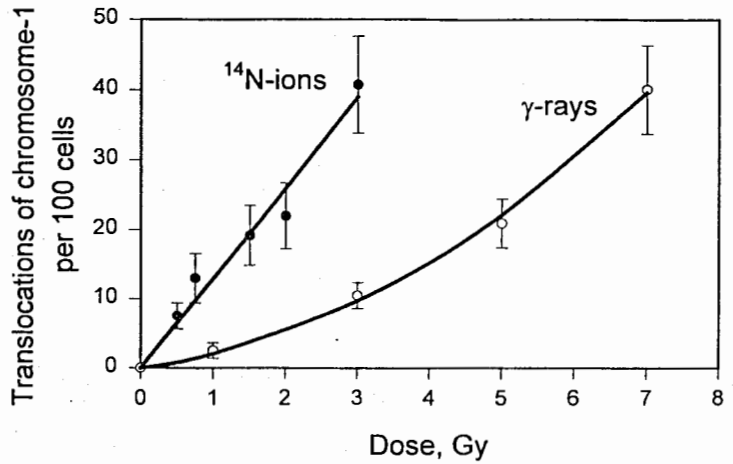

Fig 5. The frequency of translocations of chromosome-1 in human lymphocytes as a function of γ-ray and nitrogen ion radiation doses.

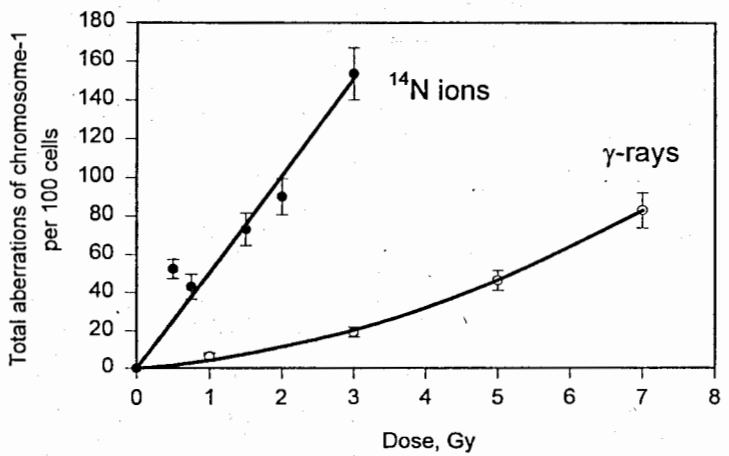

Fig. 6. The number of total aberrations of chromosome-1 in human lymphocytes as a function of γ-ray and nitrogen ion radiation doses.

The dose-response dependencies for the cells with chromosomal aberrations and for the translocations and aberrations of chromosome-1 after γ–ray and heavy ion irradiation, represented in Figs. 4–6, show the high biological effectiveness of nitrogen ions. The coefficients of RBE for the number of cells with aberrations and on the frequency of chromosome-1 translocations were ~ 3. More higher RBE was found on the criteria of the total number of aberrations (~ 4). The similar dependencies on the induction of unstable chromosomal aberrations by heavy ions in human lymphocytes and mammalian cells were shown also by using the usual microscopic technique [4, 6].

THE FINAL REMARKS

There is a great interest to elaborate a simple and efficient method for measurement of stable chromosomal aberrations induced by radiation with different physical characteristics. The development of FISH technique for chromosome staining has significantly solved this problem. Obviously, the detailed study of dependence of an induction of stable chromosomal damages from the dose of ionizing radiation, energy of particles, their LET is the important task for the decision of questions connected with forecasting of radiation safety of long space flights.

The frequencies of different types of aberrations as a function of γ–radiation dose found in our experiments are in good agreement with results obtained by others [7–8]. The observed translocation frequency in our work is higher than the dicentric frequency at all doses of γ-rays and accelerated nitrogen ions. It is in accordance with data of other authors [7, 9, 10]. Because of the danger of dicentrics underestimation at evaluation of chromosome aberrations in irradiated cells and rapid elimination of cells containing this kind of damage with increasing time after exposure [11–13] it would be better to use

the translocations as a biological dosimeter instead of dicentrics used at present time. The frequencies of radiation-induced translocations are higher than dicentrics' and therefore it is possible to detect lower doses of ionising radiation.

In experiments with heavy ions it has been shown that effectiveness of heavy charged particles on the induction of stable chromosomal abberrations is more higher than γ–rays'. The linear dose-response dependence on the induction of translocations was found after heavy ion irradiation. These circumstances can be important for using the FISH method as a way for detection of low doses of galactic space radiation for long space flights. In the last years some authors also consider the detection of the stable aberrations as a perspective way for biological dosimetry [8, 9, 14, 15].

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Красавин Е.А. и др. E19-97-170

Цитогенетические эффекты тяжелых ионов в лимфоцитах человека

Оценка генетического риска галактических тяжелых заряженных частиц является одной из важнейших задач космической радиобиологии. Цитогенетический анализ лимфоцитов крови космонавтов после космических полетов продолжительностью от 117 до 515 дней выявил увеличение частоты разных типов нестабильных хромосомных аберраций. Этот эффект может отражать действие излучений космического пространства и прежде всего Галактического космического излучения на генетические структуры. В связи с этим становится важным изучение особенностей индукции стабильных хромосомных аберраций в лимфоцитах человека разными видами ионизирующих изучений. Для анализа хромосомных повреждений после облучения ионами азота (50 МэВ/нуклон) и γ-лучами ^{137}Cs была использована FISH-техника (fluorescence *in situ* hybridization) с пробой, специфичной для ДНК хромосомы-1. Показано, что число клеток с аберрациями хромосомы-1 увеличивается и достигает 75 % после облучения ионами азота и γ-лучами в дозах 3 и 7 Гр, соответственно. Общее число аберраций и частота транслокаций хромосомы-1 увеличивались нелинейно с дозой γ-лучей, а после воздействия тяжелых ионов зависимость эффектов от дозы имела линейный характер. В экспериментах с тяжелыми ионами выявлена высокая частота делений хромосомы-1. ОБЭ ионов азота составила ~3 по частоте транслокаций хромосомы-1.

Работа выполнена в Отделении радиационных и радиобиологических исследований ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна, 1997

Krasavin E.A. et al. E19-97-170

Cytogenetic Effects of Heavy Ions in Human Lymphocytes

One of the important tasks in space radiobiology is the evaluation of the genetic risk of galactic heavy charged particles. Cytogenetic analysis of the cosmonauts' lymphocytes after space flight missions (the duration of missions was 117—515 days) reveals the increase of the frequency of different types of unstable chromosomal aberrations. This effect can reflect the action of space radiation and foremost the galactic space radiation on the genetic structures. In this connection it is important to study the peculiarities of the induction of stable chromosomal aberration in human lymphocytes by different types of ionizing radiation. The technique of fluorescence *in situ* hybridization (FISH) with chromosome-1-specific DNA probe was used to analyse the chromosomal damages after irradiation by nitrogen ions (50 MeV/nucleon) and γ-rays (^{137}Cs). It is shown that the number of cells with aberrations of chromosome-1 increases and reaches 75 % after nitrogen ion and γ-ray irradiation at 3 Gy and 7 Gy, respectively. The total number of aberrations and the frequency of chromosome-1 translocations increase nonlinearly with the dose of γ-rays and linearly after heavy ion irradiation. The high frequency of chromosome-1 deletions was revealed in experiment with heavy ions. The values of the relative biological effectiveness (RBE) of nitrogen ions were ~3 on the frequency of chromosome-1 translocations.

The investigation has been performed at the Division of Radiation and Radiobiological Research, JINR.

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