Sensitivity of Peripheral Blood Lymphocytes of Pilots and Astronauts to γ-Radiation: Induction of Double-Stranded DNA Breaks

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The levels of DNA breaks before and after *in vitro* irradiation (1 Gy) of lymphocytes from 17 donors, 41 pilots, and 8 astronauts were studied by comet assay. Seventeen donors, 41 pilots, and 8 astronauts were examined. The flights augmented individual differences in the levels of DNA breaks in blood lymphocytes and in the severity of injuries inflicted by radiation exposure to lymphocyte DNA. Dispersions in the distribution of the initial levels of DNA breaks in pilots and astronauts differed significantly from the control according to Fisher's *F* test. The dispersion of distribution of the levels of double-stranded DNA breaks after *in vitro* irradiation in the group of pilots also differed significantly from the control distribution. These results necessitate evaluation of individual sensitivity to the mission conditions during medical selection.

Key Words: *DNA breaks; radiosensitivity; blood lymphocytes; pilots; astronauts*

During fights at different heights pilots are exposed to a complex of unfavorable factors: radiation, hypergravitation, vibration, hypodynamia, zero gravitation, stress, *etc.* Greater height of the mission and its longer duration augment the severity of these exposures. Numerous biomedical investigations showed that orbiting the Earth at different heights can lead to an increase in the incidence of cytogenetic disorders [3,6], tumors [1], and non-tumor diseases [5]. However, the molecular and cellular effects developing during missions and manifesting after them are little studied. One of these effects is changes in the level of DNA injuries and changes in chromatin sensitivity to extra exposure. It is hypothesized that changes in structural characteristics of chromatin and increased sensitivity of DNA to injuries create prerequisites for the development of genome instability and high risk of tumor transformations [8].

We evaluated the sensitivity of the peripheral blood lymphocytes in pilots and astronauts to *in vitro* γ-radiation by the level of induction of double-stranded DNA breaks.

MATERIALS AND METHODS

Lymphocytes were isolated from heparinized blood by centrifugation in Ficoll-verograffin density gradient (Histopaque; Sigma), washed, and resuspended in PBS (pH 7.4) to a final concentration of 1×10^6-2×10^6 cell/ml.

The level of double-stranded breaks of lymphocyte DNA was evaluated by single-cell electrophoresis (comet assay) under neutral conditions. The severity of DNA damage detected by this method is evaluated by the content of DNA migrating from the nuclear area and the distance of its migration after electrophoresis of DNA from solitary cells immobilized on agarose.

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Lymphocyte suspension (20 μl) was mixed with 0.5% low-melting agarose solution (100 μl, Type IV) in PBS (pH 7.4) at 37°C and applied onto slides coated with 1% normal-melting agarose, covered by slides, and exposed for 5 min at 4°C on a cold metal plate. After formation of agarose gel, the cover slides were removed and the resultant slides were plunged in cold (4°C) PBS.

The preparations were exposed to γ-radiation on a Luch device (Izotop; with ^{60}Co as γ-radiation source) at a dose of 1 Gy and dose power of 0.35 cGy/min.

One-two minutes after the end of irradiation the slides were transferred into cold (4°C) lyzing buffer (pH 10.0) of the following composition: 2.5 M NaCl, 100 mM Na_2EDTA, 20 mM Tris-HCl, 1% Triton X-100, and 10% DMSO and incubated for 2 h. After cell lysis, the slides were transferred into cold (4°C) Tris-borate buffer (pH 8.2; TBE, Sigma) and incubated for 20 min. Electrophoresis was carried out in TBE buffer at 1.5 V/cm voltage and 4°C for 20 min. After electrophoresis, the slides were dried and fixed in 70% ethanol for 10 min.

DNA preparations were stained with acridine orange (Sigma; 2 μg/ml in PBS, pH 7.4). DNA comets were visualized under a MIKMED-2 microscope, variant 11 (LOMO), using a videosystem on the base of a Nikon Coolpix 4500 digital camera with stimulation filter FC1-5 (400-440 nm) and locking filter 515 (515 nm). The use of locking filter allowed recording of green fluorescence characteristic of acridine orange complexes with double-stranded DNA. DNA comets were analyzed using CometScore software (TriTek Corp.). The DNA comet tail moment (Olive tail moment) was evaluated, equal to the product of the distance from the nucleus center to the density center of the comet tail and DNA percentage in the tail.

The data were statistically processed using Statistica 6.0 software.

RESULTS

The level of DNA damage, evaluated by the DNA comet method varies greatly in human cells, depending on medical history and tobacco smoking [2,4]. Hence, only healthy nonsmokers aged 30-50 years were selected for the study. Individual differences in the levels of DNA breaks were detected in the control cohort, and the parameters in one donor were beyond the 95% confidence interval (Table 1). *In vitro* irradiation of donor blood lymphocytes in a dose of 1 Gy led to a 3-fold increase in the level of DNA breaks evaluated by comet assay. The variability of the levels of DNA breaks somewhat decreased after irradiation and all the values were within the 95% confidence interval. On the whole, the results indicated adequate selection of the control cohort.

The group of pilots consisted of civil aircraft pilots flying at different heights (6000-17,000 m) with different overall flight time (390-4520 h). The distribution of DNA break levels in the cohort of pilots differed sharply from the control (Table 1). The mean initial levels of DNA breaks in the pilots were somewhat higher than in the control, but the fact that the distributions differed significantly only by the bilateral *t* test (p=0.043) indicated great variability in the cohort of pilots. The mean levels of DNA breaks were beyond the upper threshold level of 95% confidence interval for control distribution in 10 pilots, while in 1 pilot this parameter was below the threshold 95% confidence interval for control distribution. Comparison of dispersions of DNA break level distribution in the pilots and controls showed that these values differed significantly according to Fisher's *F* test (Table 1). Irradiation of pilots' blood lymphocytes in a dose of 1 Gy led to a 2.7 times increase in the DNA Olive tail moment (Table 1). No statistically significant differences in the means in comparison with the effect of irra-

TABLE 1. Parameters of Distribution of DNA Break Levels (DNA Comet Tail Moment, arb. units) in Blood Lymphocytes of Control Donors, Pilots, and Astronauts before and after *In Vitro* Irradiation

Group	*In vitro* irradiation, Gy	*M*±*SE*	Range	SD	N ex, 95%	*F*
Control (n=17)	0	6.92±0.67	3.33-14.22	2.74	1	—
	1	20.82±1.98	8.32-36.79	8.15	0	—
Pilots (n=41)	0	9.46±1.03	1.23-31.7	6.59	10	5.77, p<0.001
	1	25.39±1.36	5.53-53.0	12.52	9	2.36, p<0.05
Astronauts (n=8)	0	8.55±1.90	2.49-18.19	5.38	2	3.84, p<0.05
	1	17.56±4.06	6.28-35.16	11.50	0	1.99

Note. SE: standard error; SD: standard dispersion; N ex 95%: number of subjects with values beyond the 95% confidence interval for control group; F: Fisher's *F* test values in comparison with the corresponding dispersions for control distributions.

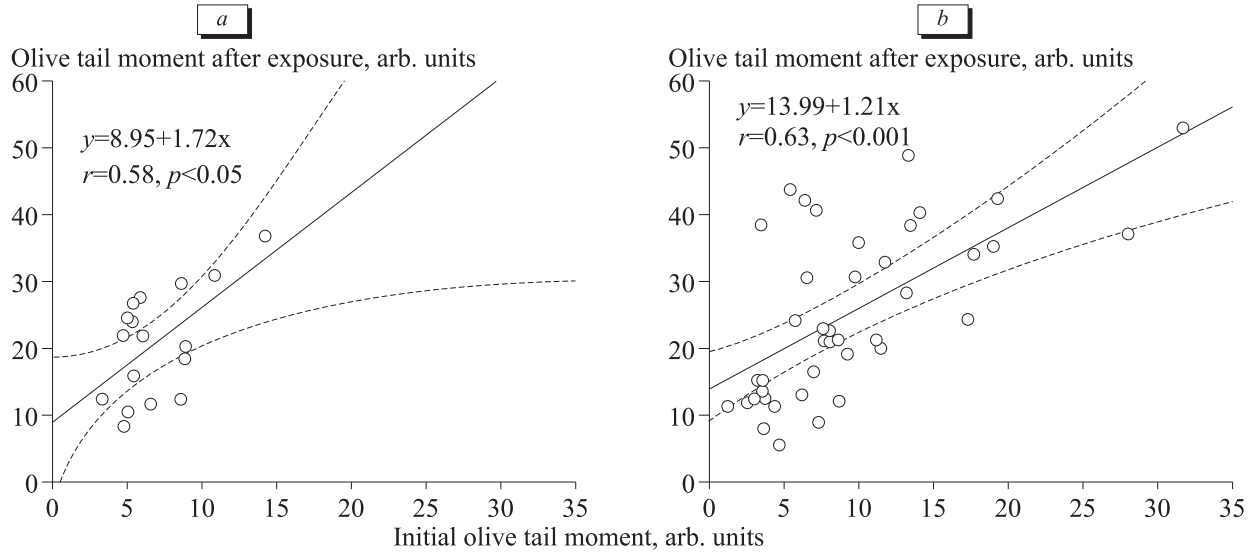

Fig. 1. Correlations between initial level of lymphocyte DNA breaks and level of DNA breaks after lymphocyte irradiation in a dose of 1 Gy in donors (*a*) and pilots (*b*).

diation in the control group were noted. The dispersions in the distribution of DNA break level in the pilots and controls after exposure to 1 Gy differed significantly according to Fisher's *F* test (Table 1).

Astronauts were examined 10-20 years after missions, the time spent in the space varying from 11 to 679 days (summary duration of several missions). Analysis of the data showed the same regularities and trends as in pilots (Table 1). However, the differences from the control were not so significant in astronauts, presumably because of a significantly lesser statistical sampling.

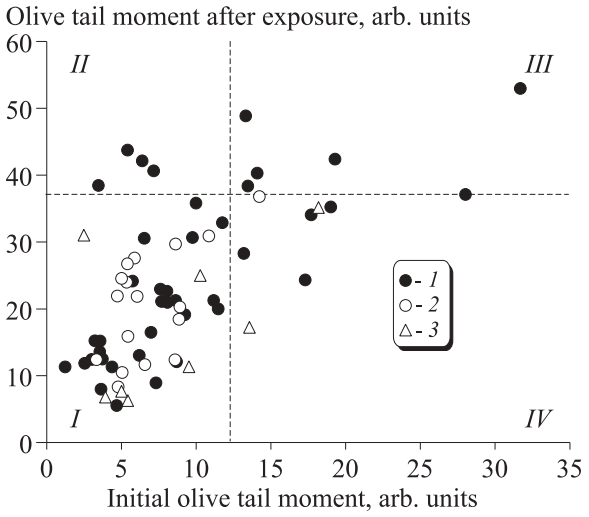

Fig. 2. Distribution of donors, pilots, and astronauts by initial levels of lymphocyte DNA breaks and levels of DNA breaks after lymphocyte exposure in a dose of 1 Gy. Intermittent lines: borders of 95% confidence interval for control group. *I*) normal; *II*) high radiation-induced damage to DNA; *III*) high radiation-induced damage to DNA after high initial level of DNA breaks; *IV*) high initial level of DNA breaks. *1*) pilots; *2*) control; *3*) astronauts.

No correlation between the duration of missions and severity of the observed effects was detected. Presumably, the increase in the dispersion of DNA break levels in lymphocytes from pilots and astronauts reflects primarily the individual reaction to unfavorable factors. For example, low-dose exposure can led to both, increase in cell resistance to subsequent exposure (adaptive response) and increase in cell sensitivity (radiosensitization) [7]. Unfortunately, through medical examinations of pilots and astronauts at present fail to detect individuals with high sensitivity to negative exposure.

Analysis of correlations revealed a statistically significant positive correlation between the initial level of lymphocyte DNA breaks and level of breaks after *in vitro* exposure to 1 Gy in control donors and pilots (Fig. 1). The presence of correlation between the initial level of lymphocyte DNA breaks and radiation-induced DNA damage indicates that in some individuals missions lead to changes in the molecular cellular parameters determining the level of initial radiation-induced DNA injuries (this can be the degree of chromatin conformation, level of gene expression and endogenous antioxidant concentrations in the immediate vicinity of DNA).

All examinees were divided into 4 groups by the initial levels of lymphocyte DNA and levels of DNA breaks after irradiation (Fig. 2). Group 1 included subjects with conditionally normal level of DNA breaks and normal radiation-induced injury to DNA. Sixteen of seventeen controls (about 94%) belonged to this group, *vs.* just 27 of 41 pilots (about 66%) and 6 of 8 astronauts (75%). Group 2 included subjects with normal initial level of DNA breaks, but with high level of DNA radiation-in-

duced injuries; it consisted of 4 pilots (about 10%). Group 3 were subjects with high radiation-inflicted damage to DNA after initially high level of DNA breaks (5 pilots; about 12%). Group 4 included subjects with initially high level of DNA injuries and low level of radiation injuries: 4 pilots (about 10%) and 2 (25%) astronauts. One control donor and one pilot were at the interface between groups 3 and 4. All individuals in groups 2-4, particularly in group 3, are in need of profound medical examinations with the use of molecular biological approaches in order to clear out the details of the mechanisms of the detected shifts.

Hence, our study showed that missions lead to a significant increase in the individual differences in the levels of blood lymphocyte DNA breaks and in the level of radiation injuries to lymphocyte DNA. The absence of correlation between the severity of these effects and duration of missions indicates the individual (diverse) reactions to flights. The disorders detected in some individuals can be early signs of disease risk and, presumably, give grounds to give up the missions.

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