STRUCTURAL GENOMIC DAMAGE IN PLUTONIUM WORKERS

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ABSTRACT

The research objective is assessment of structural genomic damages in plutonium workers. The study group included workers of the Mayak Production Association subjected to chronic occupational internal exposure to incorporated ^{239}Pu and/or external γ-rays. A lymphocyte culture of peripheral blood was chosen as an object of study. The yield of intrachromosomal exchange aberrations of chromosomal type on stained slides was analyzed using fluorescent in situ hybridization, mBAND. Linear relationships were revealed between (a) the total yield of chromosomal type aberrations (intra- and interchromosomal ones) and the absorbed dose from external exposure of the red bone marrow (RBM) to γ-rays, the absorbed dose from internal exposure of the RBM to α-radiation from incorporated ^{239}Pu, and ^{239}Pu body burden, and (b) the yield of intrachromosomal aberrations and an absorbed dose from internal exposure of the RBM to ^{239}Pu and ^{239}Pu body burden.

Keywords: chromosomal aberrations, internal radiation, ^{239}Pu, external γ-radiation.

INTRODUCTION

Both stable and unstable chromosomal aberrations in the lymphocytes of the peripheral human blood are a sensitive and easily reproducible indicator of the radiation exposure [1, 2]. Owing to the fact that T-lymphocytes are long-living cells, a significant fraction of which are retained in the human bloodstream for several decades, a lymphocyte culture of the peripheral human blood is simple and unique model for studying of the induced mutagenesis. The low spontaneous level of chromosomal aberrations in the lymphocyte culture of the peripheral blood of healthy individuals and high radiosensitivity of the lymphocytes permit to find out the increasing of the frequency of induced chromosomal aberrations over the spontaneous level even under the effect of low dose ionizing radiations.

The aim of this study was to estimate the frequency of chromosomal aberrations in the workers of the Mayak Production Association subject to occupational exposure using the mBAND method.

MATERIALS AND METHODS

The study group included 79 workers of the Mayak PA (50 men and 29 women). The main criteria for selecting the workers were as follows: (a) the 1945 – 1958 period of employment at the major factories of the Mayak PA; (b) the individual measured doses from external exposure to γ-rays and ^{239}Pu body burden. By the moment of the examination, the average age of the men was 71.1 ± 1.2 years, the age of the women was 73.7 ± 0.8 years, and the average age of the group was 72.1 ± 0.9 years. The workers of the reactor, plutonium, and radiochemical plants accounted for 44.3, 26.6, and 29.1%, respectively. The doses of external γ-exposure in the studied group varied from 0 to 3.5 Gy (the average value was 1.0 ± 0.1 Gy); the ^{239}Pu body burden was 0 – 12.3 kBq (the average value was 2.05 ± 0.37 kBq). The dose from external exposure to the RBM was within the range of 0 – 2.7 Gy (the average value was 0.86 ± 0.09 Gy); the absorbed dose to the RBM from incorporated ^{239}Pu was 0 – 0.8 Gy (the average value was 0.12 ± 0.02 Gy).

The peripheral blood lymphocytes were cultured and chromosome slides were prepared according to the standard protocol [3]. Since the proliferative activity of the lymphocytes varied significantly in different individuals and depended on the conditions of cultivation and age of the examinees and radiation dose, the cultures were incubated for 62 – 72 h at 37°C.

The mBAND hybridization of the slides was carried out according to the Xcyte lab manual MetaSystems protocol. The slides were analyzed with the help of a fluorescent microscope (using the set of DAPI, FITC, Texas Red, Spectrum Orange, DEAC, and Cy5 filters). Karyotyping was performed using the MetaSystems software (Germany).

The observed frequency of intrachromosomal aberrations in chromosome 5 was converted to the whole genome according to the content of DNA in each chromosome.

The statistical analysis of primary data was carried out by the standard method for linear regression analysis [4].

RESULTS AND DISSCUSSION

Totally, 10 977 cells were analyzed in this study. Both interchromosomal (translocations, insertions, terminal deletions) and intrachromosomal aberrations (para- and pericentric inversions,

interstitial deletions) of the chromosomal type were observed in the group of Mayak workers (79 persons).

The statistical analysis with the use of the least squares method revealed the linear relationships between the frequency of chromosomal aberrations (intra- and interchromosomal ones) in the blood lymphocytes and the absorbed dose from external γ-exposure to the RBM, the absorbed dose of internal exposure of the RBM from ^{239}Pu, and ^{239}Pu body burden.

The linear regression model contained two parameters (a and b) and represented the following dependence:

$$Y = a + bX, \quad (1)$$

where Y is the frequency of chromosomal aberrations (per 100 cells), X is the dose absorbed by an organ from external (internal) radiation (Gy) or ^{239}Pu body burden (kBq), a is the frequency of chromosomal aberrations at the «zero» dose or at the absence of ^{239}Pu in the body, and b is the frequency of chromosomal aberrations per dose unit (^{239}Pu body burden).

The results of the regression analysis presented in Table 1 were statistically significant ($p < 0.05$) for the parameters of the model and linear correlation coefficients. The significance of the regression model was estimated using the Fisher F-criterion and was $p < 0.05$. The sum of squared deviations (S^2) was an additional characteristic of the quality of the empirical data approximation of the linear dependence.

Table 1. Results of the linear regression analysis (for Y, the total yield of chromosomal aberrations)

Linear relationship	Parameters of the model		Linear correlation coefficient (r)	Sum of squared deviations (S^2)
	$a \pm \Delta a$	$b \pm \Delta b$		
Y and absorbed dose from exposure of the RBM to γ-rays	4.581±0.674	0.011±0.006	0.221	1211.63
Y and absorbed dose from exposure of the RBM to α-radiation	4.559±0.503	0.082±0.023	0.379	1101.68
Y and ^{239}Pu body burden	4.439±0.505	0.018±0.004	0.406	1075.11

As a result of the data analysis, it was found out that the frequency of chromosomal aberrations at the zero dose and zero ^{239}Pu body burden (parameter a) was 4.5 per 100 cells in all the models. This value is rather high, since, according to the literature, the background level for chromosomal aberrations in the blood lymphocytes are known to vary from 0.5 to 1.5 per 100 cells [5]. For example, A.V. Sevan'kaev concluded that the background frequency of chromosomal aberrations in the blood lymphocytes was 1.2–1.5 per 100 cells. This conclusion was made on the results of his own studies and a Japanese cohort study and carried out on large statistical material [6]. Okladnikova et al. showed that the level of chromosomal aberrations in the plutonium workers with a measured ^{239}Pu body burden lower than the sensitivity threshold of the method averaged 1.1 ± 0.2 per 100 cells. In this study the average age of the workers was 40.7 ± 0.9 years [7]. Numerous studies showed the dependence of interchromosomal aberrations (translocations) frequency from the age of an individual [8-10]. The high value of the parameter (a) in the studied group was determined by the age of the workers (the average age was 72.1 ± 0.9 years).

It should be noted that the revealed linear dependences have statistically significant but not great correlation coefficients (r) from 0.2 to 0.4. Such values of r are typical for data with a large degree of variability and, as a consequence, the prediction potential of the obtained dependences is not high. However, the correlation analysis of the primary data showed the correlation coefficients for the frequency of intrachromosomal aberrations (per 100 cells) with the absorbed dose from internal exposure to the RBM to α-radiation and ^{239}Pu body burden was equaled 0.69 and 0.76, respectively, which is two times higher than the linear correlation coefficients for the frequency of all chromosomal aberrations (see Table 1). It should be stressed that no correlation of intrachromosomal aberrations with the absorbed γ-dose to the RBM was found out ($r \approx 0$).

A regression analysis of the frequency of intrachromosomal aberrations depending on the absorbed dose from internal exposure to the RBM to α-radiation was performed. Compared to model (1), the regression model for intrachromosomal aberrations had a simpler form:

$$Z = kX, \quad (2)$$

where Z is the frequency of intrachromosomal exchange aberrations (per 100 cells), k is the parameter of the model characterizing the frequency of intrachromosomal aberrations per dose unit (^{239}Pu body burden), and X is the absorbed dose from internal exposure of the RBM to α-radiation (Gy) and ^{239}Pu body burden (KBq).

In the model (2) there is no parameter for zero ^{239}Pu body burden. This is explained by the fact that in Hande et al. the only one individual from the 11 reactor workers (the workers exposed only to external γ-radiation) had the intrachromosomal aberration ($1/1478 \times 100 \approx 0.068$) [11]. In Mitchel et al., the frequency of intrachromosomal aberrations in the control group was 0.16 ± 0.09 per 100 cells [12]. In the present study we analyzed 1172 cells of reactor workers and it was observed only one intrachromosomal aberration i.e., the frequency of intrachromosomal aberrations in the absence of internal exposure was 0.085 per 100 cells. This is a negligibly small value that was not taken into account during the regression analysis.

Table 2 shows the results of the calculations according to regression model (2).

Table 2. Results of the linear regression analysis (for Z, the yield of intrachromosomal exchange aberrations)

Linear relationship	Parameters of the model	Linear correlation coefficient (r)	Sum of squared deviations (S^2)
	$k \pm \Delta k$		
Z and absorbed dose of the RBM to α-radiation	0.60 ± 0.60	0.69	98.37
Z and ^{239}Pu body burden	0.35 ± 0.03	0.77	79.38

The results together with empirical points are shown in Figs. 1 and 2 in graphical form.

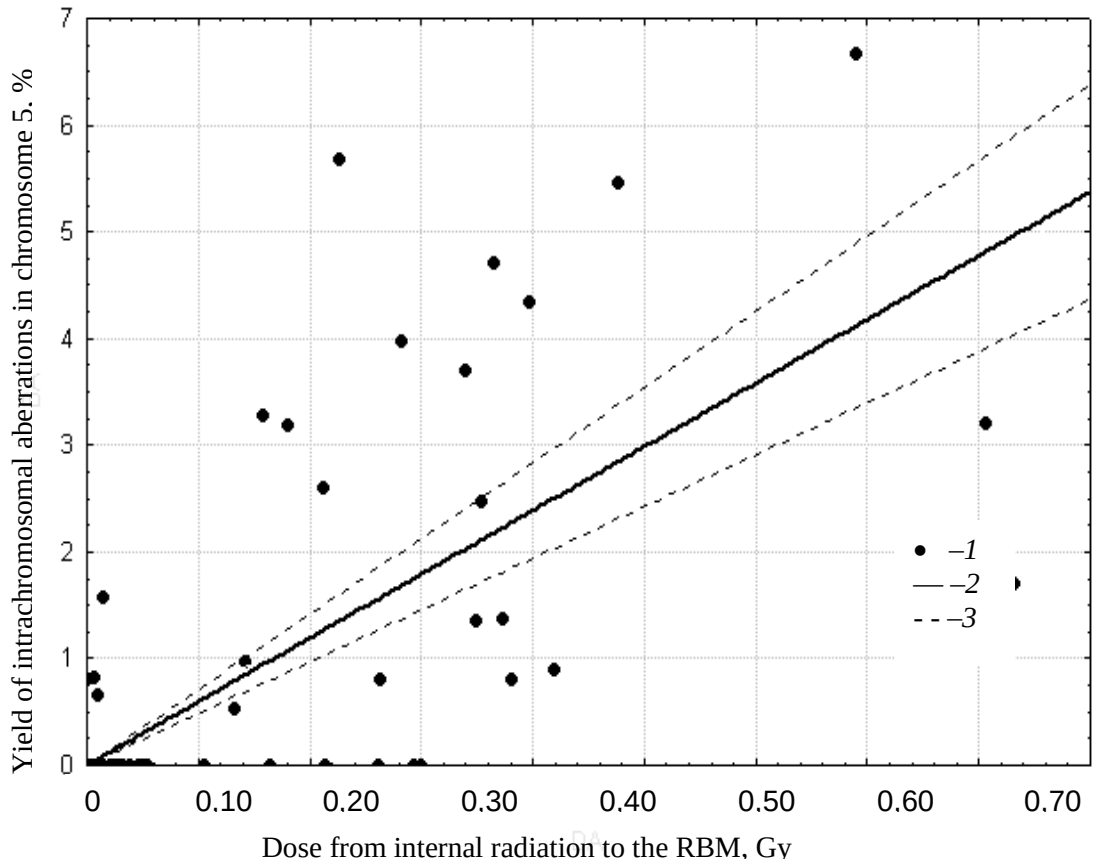

Fig. 1. Relationship between the yield of intrachromosomal aberrations and the dose from internal radiation to the RBM. (1) Experimental points; (2) regression line; (3) 95% confidence interval for the linear regression.

Since the yield of intrachromosomal aberrations to the whole genome is of some interest, this extrapolation was made taking into account the content of DNA in each chromosome. The results of intrachromosomal aberrations converting to the whole genome depending on the absorbed dose from internal exposure to the RBM from incorporated ^{239}Pu and ^{239}Pu body burden were similar to the results shown in Figs. 1 and 2 and differed only in the proportionality coefficient on the ordinate line. When extrapolated to the whole genome, the parameters of the model differed from the data presented in Table 2 by the same proportionality coefficient, and the correlation coefficients were almost identical.

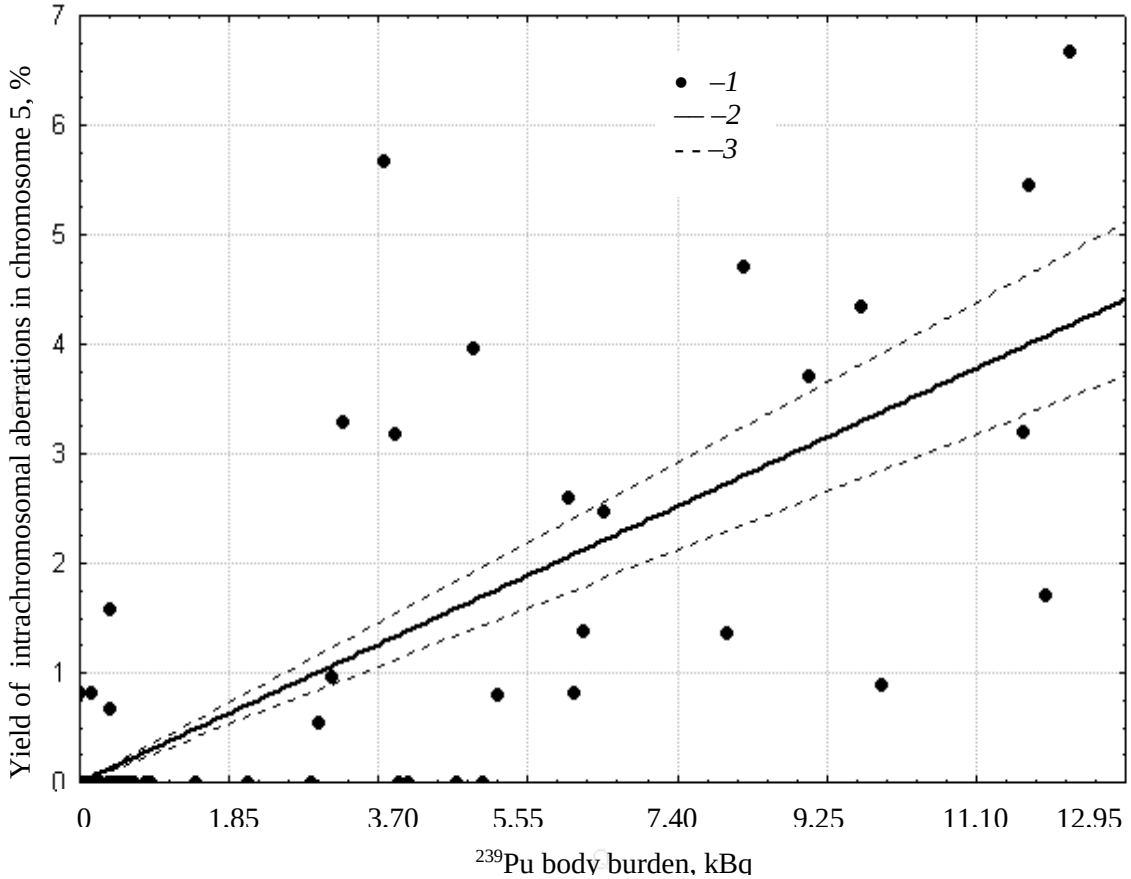

Fig. 2. Relationship between the yield of intrachromosomal aberrations and ^{239}Pu body burden. (*1*) Experimental points; (*2*) regression line; (*3*) 95% confidence interval for the linear regression.

CONCLUSIONS

The present study revealed the following linear relationships:

(1) the relationship between the total yield of chromosomal type aberrations (intra- and interchromosomal ones) and the absorbed dose from external γ-exposure to the RBM, the absorbed dose of internal exposure to the RBM from incorporated ^{239}Pu, and ^{239}Pu body burden;

(2) the relationship between the frequency of intrachromosomal aberrations and the absorbed dose of internal exposure to the RBM from incorporated ^{239}Pu and ^{239}Pu body burden.

The intrachromosomal aberrations in the lymphocytes of the peripheral blood are a specific bioindicator of internal radiation and can be used for identification of individuals who were exposed to internal α-radiation from incorporated ^{239}Pu.

REFERENCES

1. Cytogenetic Analysis for Radiation Dose Assessment. Technical Report Series No. 405, IAEA, Vienna, 2001.
2. Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies. IAEA, Vienna, 2011.
3. Burak L. E., Kodama Y., Nakano M., Ohtaki K., Itoh M., Okladnikova N.D., Vasilenko E.K., Cologne J.B., Nakamura N. FISH examination of lymphocytes from Mayak workers for

assessment of translocation induction rate under chronic radiation exposures. Int. J. Radiat. Boil. 77, 901-908 (2001).
4. Borovikov V.P. STATISTICA: art of Computer data analysis. For professionals. St. Petersburg: "Piter", 2003, 688 p. [in Russian].
5. Yarmonenko S. P. Low levels of radiation and health: radiobiological aspects. Med. Radiol. 45:5-32; 2000 [in Russian].
6. Sevan'kaev A. V. State-of-art in quantitative assessment of cytogenetic's effects in area of low doses of radiation. Radiobiologiya. V.31. N 4, pp.600-6005, 1991. [in Russian].
7. Okladnikova N. D., Osovets S. V., Kudryavtseva N. B. ^{239}Pu and chromosomal aberrations in human blood lymphocytes. Radiats. Biologiya. Radioecologiya. V. 49, N4, pp. 407-411, 2009. [in Russian].
8. Tawn E. J., Whitehouse C. A. Frequencies of chromosome aberrations in a control population determined by G-banding. Mutat. Res. 490, pp. 171-177, 2001.
9. Whitehouse C.A., Edwards A.A., Tawn E.J., Stephan G., Oestreicher U., Moquet J. E., Lloyd D. C., Roy L., Voisin P., Lindholm C., Barquinero J., Barrios L., Caballin M. R., Darroudi F., Fomina J. Translocations yields in peripheral blood lymphocytes from control populations. Int. J. Radiat. Biol. 81, pp. 139-145, 2005
10. Sigurdson A., Ha M., Hauptmann M., Bhatti P., Sram R.J., Beskid O., Tawn E.J., Whitehouse C.A., Lindholm C., Nakano M., Kodama Y., Nakamura N., Vorobtsova I., Oestreicher U., Yong L. C., Bauchinger M., Schmid E., Chung H. W., Darroudi F., Roy L., Voisin Ph., Barquinero J. F., Livingston G., Blakey D., Hayata I., Zhang W., Wang Ch., Bennett L. M., Littlefield L. G., Edwards A. A., Kleinerman R. A., Tucker J. D. International study of factors affecting human chromosome translocations. Mutat. Res. 652, pp. 112-121, 2008.
11. Hande M. P., Azizova T. V., Geard C. R., Burak L. E., Mitchell C. R., Khokhryakov V. F., Vasilenko E. K., Brener D.J. Past exposure to densely ionizing radiation leaves a unique permanent signature in the genome. Am J Hum Genet 75: 1162-1170; 2003.
12. Mitchell C. R., Azizova T. V., Hande M. P., Burak L. E., Tsokok J. M., Khokhryakov V. F., Geard C. R., Brener D. J. Stable intrachronomical biomarkers of past exposure to densely ionizing radiation in several chromosomes of exposed individuals. Rad Res 162: 257-263; 2004.