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Original Contribution

CYTOGENETIC EFFECTS IN RABBIT PERIPHERAL BLOOD LYMPHOCYTES AFTER *IN VITRO* LOW-DOSE GAMMA IRRADIATION

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ABSTRACT

The cytogenetic effect of low-dose gamma irradiation on the heredity structures of rabbits was studied. Rabbit peripheral blood lymphocytes were irradiated *in vitro* within the dose range 0.25-1.0 Gy on Rokus equipment with ^{60}Co radiation source at a dose rate of 2.22 cGy/s. The analysis of cytogenetic data showed that the frequency of induced chromosomal aberrations in rabbit peripheral blood lymphocytes, irradiated *in vitro*, augmented correspondingly to exposure dose. The dose-effect relationship was described by a linear-quadratic function of the $Y=bD+cD^2$ type. The comparison of results in the different blood donors showed a lack of significant difference in the frequency of induced aberrations in peripheral blood lymphocytes, irradiated and cultivated under the same conditions.

Key words: chromosomal aberrations, lymphocytes, low doses, gamma rays

INTRODUCTION

The global environmental pollution is responsible for the exposure of living beings to the influence of various technogenic factors, including ionised radiation The investigation on their impact upon mammalian genetic structures is especially important for evaluation of hazards related to these aspects. The exposure of cells to ionised radiation results in induction of various types of damage of DNA and cellular reparative processes (1, 2).

The exposure to high doses of ionised radiation during a short period of time produces alterations that are detectable immediately after the irradiation. They are known as threshold effects and consist in cell death, cataract, sterility, thyroid function reduction etc; the extent of damage being related to the dose rate (3, 4, 5). The low doses of ionised radiation may induce effects that could not be manifested soon after the exposure. Thus, there is not a threshold dose and each radiation absorption increases the risk for expression of these, known as non-threshold effects. They could become apparent in both irradiated individuals and their progeny (6, 7).

Despite the fact that no increase in genetic effects in irradiated human populations was observed, the results from numerous studies in experimental mammals evidence the risk of these effects, even under the influence of low doses of gamma radiation.

The possible effects of low-dose ionised radiation exposure are three types – carcinogenesis, genetic effects and *in utero* effects.

For elucidation of the mechanisms of low doses' impact, radiation-sensitive cell lines with average lethal dose (LD50) of 0.5 Gy are created, the majority of them being with apparent defects of DNA reparative systems. Regardless of this, the defects in reparative enzymes are not sufficient to explain the variety of the cellular response to ionised radiation. In mammals, peripheral blood lymphocytes are highly sensitive to radiation (8, 9). The latter provokes protein denaturation via direct ionisation on one part, and acts upon cells via radiolysis products, on the other. The determination of the influence on cells, more particularly upon DNA after exposure to low doses of gamma radiation, is especially important.

Various methods are used for detection of low-dose radiation-induced genetic effects,

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the chromosomal aberration test being one of the principal biomonitoring tests used (10, 11, 12).

The actuality of problems related to low-dose irradiation motivated the aim of the present study, i.e. the determination of the cytogenetic effect of the effect of low-dose gamma irradiation in rabbit peripheral blood lymphocytes.

MATERIAL AND METHODS

Two mature male White New Zealand rabbits aged 6 months, with equal body weight, housed and fed under the same conditions in individual cages, were used as blood donors. The blood was obtained from the ear marginal vein. The samples were processed prior to and after their irradiation (13).

Preparation of lymphocyte cultures and irradiation

Blood samples were obtained with 30 U/mL heparin as anticoagulant. Immediately after sampling, the blood was subjected to cytogenetic analysis as follows: 0.5 ml whole heparinised blood was incubated in 7 ml RPMI 1640, 3 ml heat-inactivated normal calf serum, 0.2 ml reconstituted PHA, 100E/mL penicillin, and 50µg/mL gentamicin.

The cultivation flasks were thermostated in the dark at 39^0 C. By the 24^{th} hour of the cultivation, the cultures were exposed to gamma radiation as follows:

- Group 1 – control, sham-irradiated;
- Group 2 – irradiated at 0.25 Gy;
- Group 3 – irradiated at 0.5 Gy;
- Group 4 – irradiated at 0.75 Gy;
- Group 5 – irradiated at 1.0 Gy.

Each group included two cultivation flasks from each donor.

Cultivation flasks were irradiated in a water bath (for ensuring homogeneity of the radiation source) at 39^oC. All cultures were incubated for another 24 hours. At the end of the 48^{th} hour from the beginning of lymphocyte incubation, chromosomal preparations for detection of chromosomal aberrations were prepared.

Chromosomal analysis

Chromosomal aberrations are determined after addition of colcemide at a final concentration of 0.5 µl/ml for fixation of cells at the metaphase stage (2 hours prior to end of incubation). The fixation was performed with methanol and ice-cold acetic acid (3:1). The cell suspensions were dropped onto slides and air-dried without slide warming.

Preparations were stained with the Giemsa stain.

Statistical analysis

The obtained data were statistically processed by the t-test for dependent samples, estimation of nonlinear models (Statistica software).

RESULTS AND DISCUSSION

A total number of 3000 metaphase plates with 2n=44 chromosomes (full set) were analysed. The unstable chromosomal aberrations were determined.

The data of chromosome analysis after exposure at various radiation doses are presented on **Table 1**.

The results on the number of chromosomal aberrations in rabbit peripheral blood lymphocytes showed that the exposure to 0.25 Gy already resulted in the appearance of dicentric chromosomes in both donors. With increase of applied dose, the frequency of dicentrics also increased. The number of detected acentrics prevailed after irradiation at both 0.25 Gy and 0.75 Gy. After irradiation at 1.0 Gy, the dicentrics/acentrics ratio changed in favour of dicentrics.

Comparison of both blood donors

There was no significant difference in the responses of cells obtained from both donors, exposed to equal doses and cultivated under equal conditions ($p>0.05$).

The dynamics of changes in chromosomes of peripheral blood lymphocytes in rabbits irradiated within the dose range of 0.25-1.0 Gy is presented on **Figure 1** and **Figure 2**.

Dose-effect relationship

The dose-effect relationship is presented on **Figure 3**.

Linear and linear-quadratic functions were tested for determination of the dose-effect relationship. The latter corresponded to a linear-quadratic model fitting the equation $У= (1.93\pm0.75)\ X+ (3.40\pm0.87)X^2$

P=0.03 for coefficient *b*, P=0.004 for coefficient *c*;

The radiosensitivity of peripheral blood lymphocytes has been investigated by numerous authors (14,15,16). The percentage of radiation-induced chromosomal aberrations varied not only depending on the dose rate and the post-irradiation period, but also depending on the object of experimentation (17, 18, 19).

Table 1. *Chromosomal aberrations in rabbit lymphocyte, irradiated within the dose range 0.25-1.0 Gy*

Dose	*Blood donor №*	*Number of meta-phases*	*Dicen-trics*	*Dicen-trics %*	*Acent-rics*	*Acentrics %*	*Abnormal cells*	*Abnor-mal cells %*
0 Gy	1	300	0	0	2	0.66	2	0.66
0 Gy	2	300	0	0	3	1	3	1
0.25 Gy	1	300	3	1	5	1.66	4	1.33
0.25 Gy	2	300	2	0.66	4	1.33	5	1.66
0.5 Gy	1	300	5	1.66	5	1.66	7	2.33
0.5 Gy	2	300	7	2.33	6	2.0	9	3
0.75 Gy	1	300	8	2.66	11	3.66	13	4.33
0.75 Gy	2	300	10	3.33	13	4.33	15	5
1.0 Gy	1	300	16	5.33	13	4.33	19	6.33
1.0 Gy	2	300	17	5.66	15	5.0	20	6.66

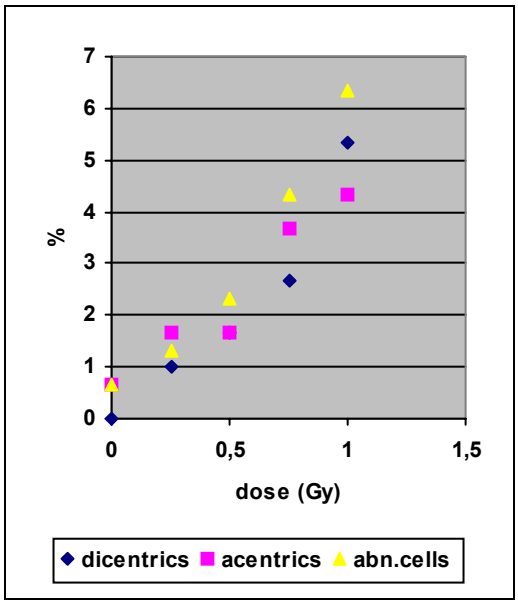

Figure 1. *Frequency of induced dicentrics, acentrics and abnormal cells in blood donor No. 1.*

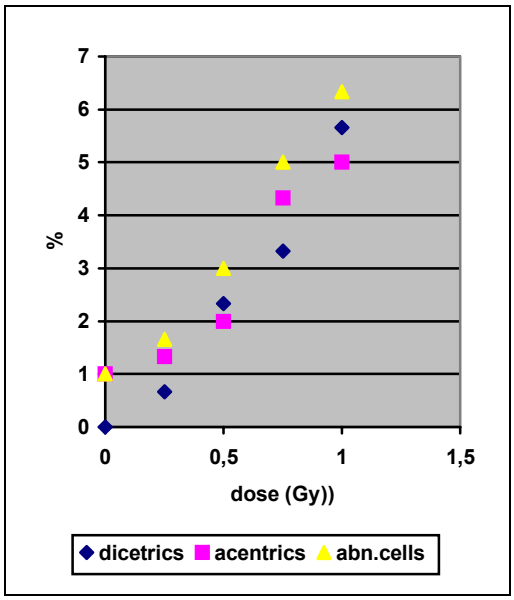

Figure 2. *Frequency of induced dicentrics, acentrics and abnormal cells in blood donor No. 2.*

According to Upton (20), the effects of exposure to low radiation doses may damage health after several years of exposure. There are evidences of association between occupational exposure, cytogenetic alterations and the increase in cancer rates (21). It is known that the probability of carcinogenesis is greater in populations exposed to radiation, since ionising radiation can raise the frequency of CA and spontaneous mutations. According to Preston (22), all tumours contain chromosome alterations; specific deletions and translocations are involved in different stages of the development of the tumour, and unspecific alterations are common in genomic instability, which is a characteristic of tumour.

Stable (balanced translocations, paracentric inversions) and unstable (dicentric and acentric fragments and rings) chromosomal aberration types are normally found after exposure to X-rays and gamma rays. The latter are generally observed as a consequence of exposure to acute ionising radiation *in vitro* or *in* vivo (23). Unstable chromosomal aberrations were not detected in the present study, which corroborates the previous statement. Stable aberrations are detected only by applying specific techniques of chromosome staining and were not detected in the present study since we carried out a conventional cytogenetic analysis. However, the possibility of there being stable type aberration in this sample cannot be discarded.

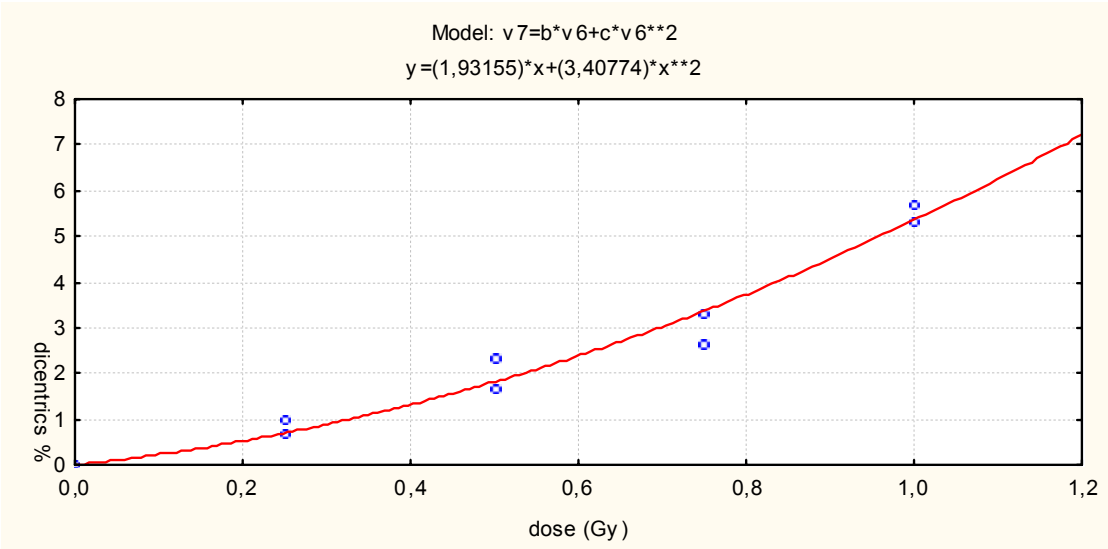

Figure 3. *Dose-effect relationship of the frequency of induced dicentrics in rabbit lymphocytes, irradiated within the dose range 0.25 -1.0 Gy.*

It is important to point out that high frequencies of chromosomal aberration in individuals occupationally exposed to genotoxic and/or carcinogenic agents may be considered a relevant biological marker to demonstrate a future cancer case (24). Thus, the increase in the number of studies in this area is necessary and important and the present study presents positive results.

In this study a linear-quadratic curve is obtained from lymphocytes irradiated *in vitro* with single doses of radiation. It is contrary to linear dose-response curves observed (25, 26, 27, 28, 29−31) in lymphocytes of patients undergoing radiotherapy (fractionated radiation schemes). The linearity is a result of the fact that the entire dose D is not applied at once, but is a sum of *n* equal fractions acting subsequently.

The test system used in the present study (frequency of cytogenetic damage in lymphocyte cultures) provided evidence for the genetic effects of low-dose irradiation in somatic cells, but is limited as a marker of induced damage of germ cells because only unstable aberrations, that either could not pass through the various stages of gametogenesis or, if having passed, could be incompatible with the development and the survival of the foetus, are detected.

CONCLUSION

The *in vitro* irradiation of rabbit peripheral blood lymphocytes within the dose range 0.25-1.0 Gy caused dose-dependent increase of unstable chromosomal aberrations – dicentrics and acentrics. The dose-effect relationship was described by a linear-quadratic function of the $Y=bD+cD^2$ type.

REFERENCES:

1. Breen AP, JA Murphy. (1995) Reaction of oxyl radicals with DNA. *Free Radical Biol. Med.* 18:1033-77.
2. Wojewodszka M., Kruszewski M., I Szumeil (1997) Effect of signal transduction inhibition in adapted lymphocytes: micronuclei frequency and DNA repair. *Int. J Radiat Biol*. 71: 245-52.
3. Lee J, Richburg JH, Shipp EB, Meistrich ML, Boekelheide K. The Fas system, a regulator of testicular germ cell apoptosis, is differentially up-regulated in Sertoli cell versus germ cell injury of the testis. *Endocrinology* 1999; 140:852-858
4. Sayama K, Moriyama T, Matsuzawa A. Marked difference in sensitivity to gamma-ray-induced cataract between BALB/c and CBA-C3H strain mice. *Jikken Dobutsu*. 1991 Oct. 40(4):541-3. Japanese.
5. Zheng R, Dahlstrom KR, Wei Q, Sturgis EM. Gamma radiation-induced apoptosis, G2 delay, and the risk of salivary and thyroid carcinomas--a preliminary report. *Head Neck*. 2004 Jul. 26(7):612-8
6. Slovinska L, Elbertova A, Misurova E. Transmission of genome damage from irradiated male rats to their progeny. *Mutat Res*. 2004 Apr 11; 559(1-2):29-37.
7. Pelevina II, Gotlib VIa, Kudriashova OV, Antoshchina M.M. Long-term effects of irradiation in the progeny of mammalian

cells. *Ontogenez*. 2001 Jan-Feb;32(1):51-7. Russian.
8. Szumiel I. Monitoring and signaling of radiation-induced damage in Mammlian cells. *Rad. Res.,* 150, (1998): S92-S101.
9. Goes E.G., Borges J.C., Covas D.T., Orellana M.D., Palma P.V., Morais F.R., Pela C.A. Quality control of blood irradiation: determination T cells radiosensitivity to cobalt-60 gamma rays. *Transfusion.* 2006 Jan; 46(1):34-40.
10. Thierens H, Vral A, de Ridder L, Touil N, Kirsch-Volders M, Lambert V, Laurent C. Inter-laboratory comparison of cytogenetic endpoints for the biomonitoring of radiological workers. Int *J Radiat Biol*. 1999 Jan; 75(1): 23-34.
11. Wolf G, Pieper, Obe G. Chromosomal alterations in Peripheral lymphocytes of female cabin attendants. *Int J Radiat Biol* 1999; 75:829-36.
12. Sasaki MS, Use of lymphocyte chromosome aberrations in biological dosimetry: Possibilities and limitations. In: Ish~hara T and Sasaki MS, eds. Radiotion- induced chromosome damage in man. New York: Alan R. Liss, 1983:585-604.
13. Hungerford, D. A., Leucocytes cultured from small inocula of whole blood and preparation of metaphase chromosomes by treatment with KCL. Speculations in *Science and Technology*. 40, 333-335, 1965.
14. Lloyd D.C., Edwards A.A. Chromosome aberrations in human lymphocytes: Effect of radiation quality, dose and dose rate, Radiation-Induced Chromosome Damage in Man (Ish~hara, T., Sasaky, M.S., Eds), Alan R. Liss, New York. 1983:23-49.
15. Bauchinger M. Quantification of low-level radiation exposure by conventional chromosome aberration analysis. *Mutat Res* 1995. 339:177-89.
16. Omar F. Garcia, Adriana T. Ramalho, Mariana Di Giorgio,Sylvia S. Mir, Marco E Espinoza, J. Manzano, N. Nasazzi, I. Lopez, Intercomparison in cytogenetic dosimetry among five laboratories from *Latin America Mutat Res* 1995; 327:33-9.
17. Obralic N., Sofradzija A. [Cytogenetic dosimetry of gamma rays on human lymphocytes. *Med Arh.* 1994;48(3):105-7. Croatian.
18. Okladnikova N.D., Pesternikova V.S. The chromosome aberrations in human peripheral blood lymphocytes 43-46 years after the acute radiation disease. *Radiats Biol Radioecol.* 2005 May-Jun. 45(3):287-90. Russian.
19. Das B.C., Sharma T., Blood lymphocyte culture system: quantitative analysis of X-ray induced chromosome aberrations in man, muntjac and cattle. *Mutat res* 1983 110. 1. 111-39.
20. Upton AC. The biological effects of radiation. Sc. Amer. 1982;(246): 41.
21. Mitelman F. Catalog of chromosome aberrations in cancer. 5th ed. New York: Wiley; 1994.
22. .Preston RJ. Short/medium term carcinogenicity tests and genetic and related effects. IARC Meeting – Lyon; 1997.
23. Hagelström AH, Gorla NB, Larripa IB. Chromosomal damage in workers occupationally exposed to chronic low-level ionising radiation. *Toxicol Lett* 1995;(76): 113-7.
24. Sorsa M, Ojajarvi A, Salomaa S. Cytogenetic surveillance of workers exposed to genotoxic chemicals: preliminary from a prospective cancer study in a cytogenetic cohort. *Teratog Carcinog Mutagen* 1990. (10):215-21.
25. Fenech M, Denham J, Francis W, Morley AA (1990) Micronuclei in cytokinesis-blocked lymphocytes of cancer patients
26. Hofer E.P., Brucher S., Mehr K., Tibken B. (1995) An approach to a biomathematical model of lymphocytopoiesis. *Stem Cells* 13. S1:290300
27. le Roux J., Slabbert J, Blekkenhorst G .(1998) Assessment of the micronucleus assay as a biological dosimeter using cytokinesis-blocked lymphocytes from cancer patients receiving fractionated partial body − . radiotherapy. *Strahlen Onkol* 174:75−81
28. Lee T.-K., O'Brien K.F., Naves J.L. *et al.* (2000) Micronuclei in lymphocytes of prostate cancer patients undergoing radiation therapy. *Mutat Res* 469:63−70
29. Thierens H., Vral A., Eijkeren M. van, Speleman F., Ridder L. de (1995) Micronucleus induction in peripheral blood lymphocytes of patients under radiotherapy treatment for cervical cancer or Hodgkin's disease. *Int J Radiat Biol* 67:529−539
30. Venkatachalam P., Solomon F.D.P., Prabhu BK, Mohankumar MN, Gajendiran N, Jeevanram RK (1999) Estimation of dose in cancer patients treated with fractionated radiotherapy using translocation, dicentrics and micronuclei frequency in peripheral blood

lymphocytes. *Mutat Res* 429:1−12
31. Vorobtsova I., Darroudi F., Semyonov A. *et al.* (2001) Analysis of chromosome aberrations by FISH and Giemsa assays in lymphocytes of cancer patients undergoing whole-body irradiation: comparison of *in vivo* and *in vitro* irradiation. *Int J Radiat Biol* 77:1123−1131.