Exp Oncol 2013
35, 1, 65–68

Experimental ONCOLOGY

EXPERIMENTAL VALIDATION OF PREVENTION OF THE DEVELOPMENT OF STOCHASTIC EFFECTS OF LOW DOSES OF IONIZING RADIATION BASED ON THE ANALYSIS OF HUMAN LYMPHOCYTES' CHROMOSOME ABERRATIONS

E.A. Domina, V.F. Chekhun

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine

***Aim*: On the basis of the cytogenetic research, to develop and validate the strategy of the measures to prevent the stochastic effects of low-doses radiation on humans. *Methods*: Test system with human peripheral blood lymphocytes, metaphase analysis of chromosomal aberrations was used. Cells were cultured according to the standard procedures with modifications. The analysis of painted chromosome preparations was carried out according to the conventional requirements to metaphase spread. *Results*: The experimental material, obtained on chromosomal level of radiosensitive cells, concerning validation of prevention strategy of stochastic effects of low doses of ionizing radiation, primarily cancer, is discussed. Its key phases are the following: estimation of individual radiosensitivity, accounting of the co-mutagens influence and use of effective atoxic radioprotectors. The practicability of the primary prevention strategy of radiogenic cancer has been evidence based, especially in case of the influence of small doses of ionizing radiation. Cytogenetic studies using G_2-radiation sensitivity assay are essential component of priority populations' health monitoring for formation high cancer risk groups and implementation developed strategies of stochastic effects prevention, including radiogenic cancer, among persons with known hypersensitivity to ionizing radiation. It applies the nuclear industry workers, medical staff (radiation oncologists, radiologists), and priority populations living in areas contaminated with radionuclides. *Conclusion*: Strategy for the prevention of stochastic effects of low-doses radiation, especially cancer risk, is elaborated on the cytogenetic studies basis, implies that cancer risk reduction is provided by assessment of individual radiation sensitivity (G_2-radiation sensitivity assay), by taking into account the additional effect of co-mutagens, and with the use of non-toxic effective radioprotectors.**

***Key Words*: prevention, stochastic effects, radiation sensitivity, cytogenetic effect, co-mutagens, radioprotectors.**

The stochastic effects of low doses of ionizing radiation also include chromosome aberrations and cancer, the incidence of which is a probabilistic process and does not have dose threshold [1]. It was found that the effect of irradiation on the process of carcinogenesis at low doses may be greater per dose unit than larger doses. Researchers attribute this to less expression of apoptosis, reparation, change of the irradiated cells sensitivity to the action of other carcinogenic factors in comparison with the effects of large doses of radiation, reduction of the organism's compensatory and recovery capabilities, etc. [2–5].

The problem of radiogenic cancer has become especially actual and has obtained global scale in connection with the accident in Chernobyl Nuclear Power Plant (April 1986) and Fukusima-1, Japan (March 2011), what has pointed out that nuclear reactors have no absolute guarantee of safe operation.

To date there is no theory on how to predict the development of stochastic effects, including radiation-induced cancer, and choose the means of its prevention. In most academic oncological centers of CIS the research paradigm is focused on creation and improvement of curative methods, on the problem and investigation of cancerogenesis, on early detection of diseases. However, not enough attention has been paid to primary prevention of cancer, including the one of radiation genesis.

Received: February 10, 2012.

*Correspondence: E-mail: edjomina@ukr.net

Abbreviations used: HPBL – human peripheral blood lymphocytes; IRS – individual radiation sensitivity; RAR – radioadaptive response.

Using the modern knowledge about the role of the genetic factor in the development of oncologic diseases is a potentially fruitful area of individual prevention of this disease. The inherited genetic susceptibility, genomic instability, mutation modifications in proto-oncogenes and suppressor genes, dynamic research of chromosomal aberration level and spectrum etc., are the key genetic factors indicating to high carcinogenic risk. The results of the researches of genetic susceptibility to irradiation effect show sufficiently high risk of deterministic and stochastic effects development, which the individuals with radiosensitive genotype have, especially in case of low radiation influence. This is crucial in the case of medical examination of employees of companies with high carcinogenic risk conditions.

By now it has been accumulated the data, which show the relationship of mutagenesis in somatic cells with carcinogenesis, and chromosomal aberrations are sensitive indicator of radiation exposure on the human organism. This allows the use of cytogenetic indicators as prognostic markers of the oncological pathology risk development [6].

According to current views, the radiation-induced destabilization of human genome is potentially oncogenic [7]; and human peripheral blood lymphocytes (HPBL) (T-lymphocytes) are unique research object with special characteristics being an object of radiation and cytogenetic research [8]. The basic premise for this is a high chromosomal radiosensitivity of lymphocytes comparing to the chromosomes of other cells as *in vivo* as *in vitro*, which allows us to register indubitable increase of induced chromosomal aberrations level

compared with spontaneous one, in low levels of irradiation. A high mobility of lymphocytes in blood stream, the distribution of lymph nodes all over the body, the ability of lymphocytes to accumulate chromosome damages not only make it possible to draw conclusions about the radiosensitivity of human organism as a whole, but to prognosticate the consequences of irradiation. In the radiation cytogenetics guidelines there are numerous statements about approximately equal outlet of chromosomal aberrations in case of lymphocytes irradiation *in vivo* and *in vitro*; that means that the cells respond to the irradiation as autonomic biological system [8, 9].

Taking into account the importance of the problem of negative biomedical effects of small exposure doses, this study presents a strategy of their prevention, which is argued by cytogenetic studies data. The proposed prevention strategy includes the following key stages: estimation of individual radiosensitivity, accounting of the co-mutagens influence and use of effective atoxic radioprotectors.

MATERIALS AND METHODS

We used test system with HPBL, metaphase analysis of chromosomal aberrations. The protocol for study was approved by local Ethics Committee.

Analysis of aberrations level and spectrum in chromosomes of HPBL, which are acknowledged to be one of the most sensitive to radiation and are recommended WHO and UNSCEAR for biological indication of the radiation injury of human organism [10], gives an objective information about genome integrity in human somatic cells.

Lymphocytes cultures. Cells were cultured according to the standard procedures with modifications [11]. Cells were incubated in RPMI 1640 medium, containing 0.1 μg/ml PHA (M form, Gibco-Invitrogen) for 52 h (last 3 h with colcemid). This procedure made it possible to analyze cells in the first post-radiation mitosis. The analysis of painted chromosome preparations was carried out according to the conventional requirements to metaphase spread [12].

Statistical analysis. Cytogenetic parameters obtained were analyzed by the means of standard descriptive and variation statistics and included calculation of mean group values (M), standard error (SE), standard deviation (SD), sample dispersion (s^2), coefficient of variation (CV) etc. and representing experimental data distributions as histograms. After analysis of their forms and fitting of obtained functions to normal ones 95% confidential intervals were determined as M±, 96SD. Radiosensitive cut-off point was also calculated as the 90^{th} percentile of obtained G_2 scores. F-test was applied to indicate significance of the differences between donors. A significance level of $p < 0.05$ was used throughout.

RESULTS AND DISCUSSION

Assessment of human organism individual radiation sensitivity (IRS) which makes it possible to prognosticate the risk for pathological radiogenic condition.

According to the modern views, cells sensitivity to the influence of ionizing irradiation is formed by a complex of factors: on the one hand, the particularities of genetic structure and conformation of DNA, the level of endogenous protectors, antioxidant activity, characteristics of cell cycle, intensity of apoptosis, regulation of proliferation processes, effectiveness of reparation system etc.; on the other hand, the level of integral absorbed radiation dose and its distribution in time and space, terms after irradiation, as well as the character of influence combination with other environmental factors. In case of assessment of general human radiation sensitivity the individual differences are neutralized. However, in case of equal dose of irradiation the large amplitude of IRS values means that high variability is observed. In case of high stress intensity human individual characteristics do not play a crucial role, as the damage exceeds protective and compensative abilities of the organism. The modern point of view on etiology of radiogenic cancer is a dominant carcinogenic danger of the influence of low doses of irradiation. Moreover, most of radiation effects of technogenic sources are characterized by low doses and low power of doses. That's why the definition of IRS is especially important in the range of influence of low doses of ionizing radiation [13–15]. From this point of view it is recommended to use G_2-radiation sensitivity assay, which we designed on the basis of classical theses of radiation cytogenetics, in order to identify individuals with high IRS in a healthy cohort [16].

According to the developed algorithm (Figure), the IRS determination of relatively healthy individuals is advisable to carry out under the following conditions:

- testing γ-irradiation of HPBL cultures should be done in the most radiosensitive period of the first mitotic cycle — late G_2 (46 h of cells incubation);
- dose of γ-irradiation is 1.5 Gy at power 1.0 Gy/min, which allows to identify the maximum variability of the IRS indicators;
- cell culture fixation for 52 h of incubation takes into account radiation-induced mitotic delay and provides metaphase analysis of aberrations of chromatid type (deletions), that are dominant in the injury spectrum of G_2-period (Fig. 1).

For practical use of cytogenetic G_2-factor test it was proposed the coefficient of IRS (C_{IRS}), which is the ratio of:

$C_{IRS} = M_{IRS}/M$, where

M_{IRS} — the total frequency of chromosomal aberrations in individual HPBL;

M — the range of normal values for this indicator ($M \pm 1{,}96\ \sigma$).

If for hypersensitive persons the radiation-induced cytogenetic effect with the same radiation dose exceeds the upper bound of the range of normal values variability, the ratio is always > 1, whereas in hyposensitive persons it will be < 1.

This method allows to estimate genetically determined sensitivity of the individual to the radiation factor.

As chromosomal modifications development in cell population is considered to be potentially oncogenic [17], then the increase of radiation sensitivity comparing to its average population values is a risk factor of radiation carcinogenesis. In this connection we have developed the indications for cytogenetic examination of the individuals who are working (or who are going to work)

in the range of action of ionizing radiation, as well as for other priority categories of the population.

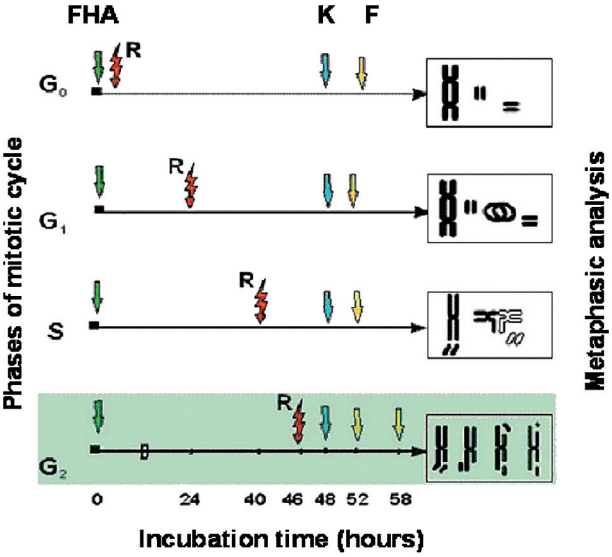

Figure. Algorithm of individual radiosensitivity detection in practically healthy individuals by cytogenetics alterations in peripheral blood lymphocytes culture. FHA — phytohemagglutinin; R — gamma-irradiation in dose equal to 1.5 Gy; K — colcemid; F — terms of cell culture fixation

Accounting of the co-mutagens influence. These are the substances which, not having own intrinsic mutagenic properties, can considerably modify (intensify) the effects of well-known mutagens, including radiation-induced effects of low doses. Particular danger in this case present such medicines as calcium antagonists (verapamil, intensifies bleomycine action), ascorbic acid (intensifies the actions of hydrogen hydroperoxide or bleomycine), caffeine (increases cytogenetic effects of methotrexate) etc. In spite of the fact that there are some data showing the possibility of co-mutagen modification of induced mutagenesis, this problem as a whole is not given appropriate consideration. The issue on possible role of the regulation of reparation processes in co-mutagen effects formation in human cells still remains open, but theoretically it can be related to the inhibition of these processes [18]. We have shown that such co-mutagen as caffeine (⩾ 200 μg/ml of blood) 5.7-fold increases the radiation-induced cytogenetic effect, without influencing spontaneous level of chromosome aberration in human somatic radiosensitive cells. This effect is due to the aberrations of chromosomal type, predominantly paired fragments and dicentric chromosomes [19]. Presented data indicate that individuals with determined increased IRS should be encouraged to limit consumption of substances with known co-mutagen effect.

Use of effective atoxic radioprotectors. These are the substances given prior to irradiation for reduction of its impact on organism and for human genome resistance intensification. Examples of such radioprotectors are inosine and thymalin, their action is due to activation of enzymatic reparation processes.

Inosine, substance of nature origin, being precursor of ATP and nucleotides synthesis, maintains energy balance in cells of different tissues, possesses antihypoxic properties, and stimulates reparation as well as different metabolic processes. We have shown that inosine reduces the level of chromosome aberrations induced in LPB of healthy individuals in the range of low-doses γ-irradiation *in vitro* to the values of spontaneous aberrations, so playing the role of radioprotector for cells. The most pronounced effect of inosine in the preventive dose (estimated at 0.01 mg/ml of blood) is observed at the lowest irradiation doses 0.1 — 0.2 — 0.3 Gy (Table).

Table. Modification of radiation-induced cytogenetic effects in lymphocytes of peripheral blood of healthy individuals under the inosine influence (mean group values)

Dose, Gy	Cytogenetic indicators (per 100 metaphases analyzed)					
	Frequency of aberrant metaphases, %	Total frequency of chromosome aberrations, %	Aberration of chromosomal type, %	Dicentrics, %	Aberrations of chromatide type, %	Deletions, %
0.1	6.06 ± 0.6	6.06 ± 0.6	2.99	0.3	3.07	3.0
In + 0.1	1.3 ± 0.1	1.66 ± 0.1	1.06	0.2	0.6	0.6
0.2	7.0 ± 1.6	7.06 ± 1.6	3.26	0.5	3.8	3.6
In + 0,2	2.6 ± 0.4	2.7 ± 0.4	1.6	0.5	1.1	0.75
0.3	7.5 ± 1.0	7.76 ± 1.0	4.16	0.9	3.6	3.4
In + 0.3	2.2 ± 0.6	2.2 ± 0.6	1.2	0.2	1.0	0.8
0.5	10.9 ± 1.4	11.3 ± 1.4	5.23	1.3	5.9	5.6
In + 0.5	3.5 ± 1.0	4.5 ± 1.0	3.5	1.5	1.0	0.5
1.0	17.4 ± 1.5	18.6 ± 1.8	11.4	5.4	7.2	6.8
In + 1.0	14.8 ± 1.1	15.3 ± 1.0	8.6	4.2	6.7	6.0

Note: In – inosine.

In this dose range the level of radiation-induced chromosome aberrations reduces, reaching values of average population level of spontaneous genetic alterations in HPBL. The coefficient of modification of radiation effects is 3.8 (± 0.2) — 2.7 (± 0.1) — 3.5 (± 0.1), respectively. With further dose increase to 1.0 Gy radioprotective effect of inosine reduces, and coefficient of modification is, respectively, 1.2 [20].

While searching means capable to restore cells from radiation-induced changes, it was found that agents of thymus origin, including thymalin, may be effective for these purposes, as targets for their action are just human lymphocytes.

Thymalin is a complex mixture of biologically active substances, mainly peptides, isolated from the mammals thymus tissues. It refers to medicines that increase genome stability, activate immune and repair systems. Thymalin in prophylactic dose (estimated at 0.002 mg/ml of blood) has radioprotective effect on the genetic apparatus of HPBL. At a dose of 0.2 Gy his effect is reduction of chromosomal aberrations incidence from 5.0 ± 1.3% to 2.0 ± 0.9%, and at a dose of 0.5 Gy — from 8.0 ± 1.0% to 4.0 ± 1.0%, i.e. twice. In the low-dose range under thymalin impact the ray markers — dicentric chromosomes — disappeared. The observed radioprotective effect of thymalin is due to its stimulating effect on the repair of primary radiation damages in the first period of the intermolecular test, that is, on the border of the periods of G_1/S of mitotic cycle [21].

Recommended drugs will exhibit radioprotective properties in the best way when used on the background of vitamin supply as complementary approaches to the protection of the human genome from the mutagenic effects of low doses of ionizing radiation.

In the implementation of measures for radiogenic cancer prevention, based on this strategy, we rec-

ommend to take into account also information in the patient's history concerning precancerous conditions, the set of environmental factors, lifestyle, including adverse health habits, unbalanced nutrition, etc.

Cytogenetic studies using G_2-radiation sensitivity assay are essential component of priority populations' health monitoring for formation high cancer risk groups and implementation developed strategies of stochastic effects prevention, including radiogenic cancer, among persons with known hypersensitivity to ionizing radiation [22]. Above all, it applies the nuclear industry workers, medical staff (radiation oncologists, radiologists), and priority populations living in areas contaminated with radionuclides.

To be fair it should be noted that researchers' attention recently is directed at finding correlations between human organism's resistance to radiation and lymphocytes' ability to generate radioresistance induced by ionizing radiation — radioadaptive response (RAR) [23–25]. It was shown that the RAR formation can reduce cancer risk at low-doses radiation [26]. However, in some studies it was found that in various population groups, affected by the Chernobyl accident, the ability of lymphocytes to form RAR is reduced or even absent [27]. We have evident data of clinical and cytogenetic survey of 17 thousand liquidators of the Chernobyl NPP accident, which indicate that low-doses of ionizing radiation are statistically significant factors for the increased cancer risk [4]. Therefore, we propose the method of prevention of the development of stochastic effects of radiation is directed at grading/minimization effect of small doses.

In conclusion, strategy for the prevention of stochastic effects of low-doses radiation, especially cancer risk, is elaborated on the cytogenetic studies basis, implies that cancer risk reduction is provided by assessment of IRS (G_2-radiation sensitivity assay), by taking into account the additional effect of co-mutagens, and with the use of non-toxic effective radioprotectors.

REFERENCES

1. Hrodzynskyi DM. Radiobiology. Kyiv: Lybid, 2011. 448 p. (in Ukrainian).

2. Twenty five years after the Chernobyl Accident. Security of the future. National Report of Ukraine. Kyiv: KiM, 2011, 368 p. (in Russian).

3. Akhmatullina NB. Long-term radiation effects and induced genomic instability. Radiation Biology. Radioecology 2005; 45: 680–7 (in Russian).

4. Domina EA. Low-dose ionizing radiation as risk factor for malignant neoplasms occurrence among Chernobyl NPP accident liquidation participants. Chernobyl Catastrophe. 20 Years Later. Greenpeace Report. Amsterdam, Greenpeace, 2006, 235–41.

5. Burlakova EB. Low-dose radiation and nanostructures. IV Intern Conf. Chronic Radiation: Low-Doses Effects. Russia, Cheliabinsk, 2010: 7–9 (in Russian).

6. Snigireva GP, Novitskaya NN, Popova GM. Value of cytogenetic tests to predict long-term effects of irradiation. Radiation Biology. Radioecology 2011; **51**: 162–7 (in Russian).

7. Sanberg AA. Chromosome abnormalities in human cancer and leukemia. Mutat Res 1991; **247**: 231–40.

8. Investigation Guidelines on Genetic Effects in Human Populations. Geneva: WHO, 1989; **46**. 122 p.

9. Sevan'kayev AV. Radiosensitivity of Human Lymphocyte Chromosomes in Mitotic Cycle. Moscow: Energoatomizdat, 1987. 160 p. (in Russian).

10. Cytogenetic Analysis for Radiation Dose Assessment. Technical Report series No 405, Vienna: Int Atom Energy Agency, 2001. 138 p.

11. Dyomina EA, Ryabchenko NM. Estimation of individual radiosensitivity of practically healthy persons of on the basis of the scheme of cytogenetic investigation. Lab Diagnostics 2006; **36**: 30–4 (in Russian).

12. International System of Cytogenetic Nomenclature for Acquired Chromosome Aberrations. Mitelman F eds. Basel, 1995. 120 p.

13. Domina EA, Druzhyna MO, Riabchenko NM. Human Individual Radiosensitivity. Kyiv: Logos, 2006. 126 p. (in Russian).

14. Lin SZ. Biological effects of low level exposures to ionizing radiation: theory and practice. Human and Experimental Toxicology 2010; **29**: 275–81.

15. Summary of low-dose radiation effects on health. UNSCEAR 2010 Report. New York: UNITED NATIONS, 2011. 106 p.

16. Domina EA, Riabchenko NM, Druzhyna MO, Chekhun VF. Cytogenetic Method (G_2-assay) of Human Individual Radiosensitivity Determining for the Purpose of Primary Prevention of Radiogenic Cancer. Methodic Recommendations. Kyiv: Ministry of Public Health of Ukraine, 2007. 28 p. (in Russian).

17. Hagmar L, Stromberg U, Bonassi S, ***et al***. Impact of types of lymphocyte chromosomal aberrations on human cancer risk: results from Nordic and Italian cohorts. Cancer Res 2004; **64**: 2258–63.

18. Durnev AD. Mutagenesis modification in human cells. Vestnik RAMN 2001; **10**: 70–80 (in Russian).

19. Grinevich YA, Domina EA. Immune and Cytogenetic Effects of Dense and Rare Ionizing Radiation. Kyiv: Zdorovya, 2006. 200 p. (in Russian).

20. Chekhun VF, Domina EA, Demchenko EN. Patent of Ukraine № 61604. The method of reducing of spontaneous and radiation-induced frequency of genetics damage in somatic non-malignant human cells. 25.07.2011. Bulletin № 14 (in Ukrainian).

21. Grinevich YA, Domina EA, Bendiukh HD. Thymalin impact on the radiosensitivity of chromosomes of peripheral blood lymphocytes of thyroid cancer patients. Oncologiya 2004; **6**: 218–21 (in Russian).

22. Chekhun VF, Domina EA. Patent of Ukraine № 67007. 25.01.2012. Method of primary prevention of radiogenic cancer. Bulletin № 2 (in Ukrainian).

23. Tapio S, Jacob V. Radioadaptive response revisited. Rad Env Biophys 2007; **46**: 1–12.

24. Matsumoto H, Takahashi A, Ohnishi T. Radiation-induced adaptive responses and bystander effects. Biol Sci Space 2004; **18**: 247–54.

25. Lin Lu, Baocheng Hu, Fang Yu, ***et al***. Low-dose radiation-induced adaptive response preventing HPRT mutation is Fhit independent. Int J Radiat Biol 2009: **85**: 532–7.

26. Sakai K. Enchancement of bio-protective functions by low dose/dose-rate radiation. Dose-Response 2006; **4**: 327–32.

27. Pelevina II, Afanasiev GG, Aleshchenko AB, ***et al***. Radiation-induced adaptive response in children and the impact