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INCREASED INDIVIDUAL CHROMOSOMAL RADIOSENSITIVITY OF HUMAN LYMPHOCYTES AS A PARAMETER OF CANCER RISK

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Aim: **Evaluation of chromosomal radiosensitivity of healthy individuals and determination those with the increased susceptibility to radiogenic cancer.** ***Methods:*** **Cytogenetic examination of radiation induced injuries in lymphocytes of healthy individuals (n = 103) was carried out on the basis of G_2-assay. Test system of peripheral blood lymphocytes with metaphase analysis was used.** ***Results:*** **On the basis of the obtained "stage-effect" and "dose-effect" calibrating curves the scheme of cytogenetic examinations of healthy individuals was developed. Analysis of cytogenetic parameters induced by G_2 irradiation at 1.5 Gy dose revealed their high interindividual variability. The highest differences were registered for chromatid type aberrations (*CV* = 42.1%) with the chromatid break predominance in the spectrum (*CV* = 37.5%). Statistical analysis of the distributions of the obtained individual cytogenetic parameters indicated 12% individuals with increased chromosomal radiosensitivity.** ***Conclusions:*** **Cytogenetic evaluation of individual chromosomal radiosensitivity based on G_2-assay has its perspectives in the formation of groups with increased risk of radiogenic cancer developing and its primary prophylactics among healthy population.**
Key Words: **individual radiosensitivity, G_2-assay, radiogenic cancer.**

The problem of new approaches to the formation of increased cancer risk groups have being an actual practical and fundamental for a long time and is closely connected with the mechanisms of cancer development and its primary prophylactics. Assessment of cytogenetic effects in human peripheral blood lymphocytes (PBL) induced by test irradiation at one of the most sensitive cell cycle stage (G_2-assay) is one of the methods used in the investigations of human individual radiosensitivity (IR). Cytogenetic methods based on chromosome aberrations analysis make it possible quantitative estimation of radiation effects on human organism taking into account its individual peculiarities and thus to estimate its IR. The main bases for application of cytogenetic methods in radiobiology are high radiosensitivity of human PBL and formation of specific radiation-induced chromosomal aberrations [1]. They are considered to be the proven markers of cancer development in the calculations of cancer risk after exposure to ionizing radiation [2]. These data are extrapolated from the known epidemiological genetic investigations carried out by European authors, who revealed reliable correlation between cancer incidents and frequency of chromosomal aberrations in human somatic cells [3].

The radiosensitivity of cancer patients, children evacuated from Chernobyl area, children after irradiation of their PBL cultures at adapting doses was determined On the basis of cytogenetic markers [4–6]. However assessments of IR in the group of healthy individuals on the basis of test irradiation of PBL in G_2 stage of cell cycle period with the subsequent analysis of chromosomal aberrations are insufficient and need further development. Their actuality is obvious as they allow objective and comprehensive prediction of potential danger of radiation effects from the point of view of human pathologies development based on genome instability (first of all cancer and multifactorial diseases).

This work presents the data obtained during the cytogenetic examinations of healthy individuals with the purpose to evaluate their chromosomal IR and using this criterion to determine those with the increased susceptibility to radiogenic cancer.

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Abbrevations used: PBL – peripheral blood lymphocytes; IR – individual radiosensitivity.

MATERIALS AND METHODS

Analysis of aberrations level and spectrum in chromosomes of human PBL, 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 [8–10], gives an objective information about genome integrity in human somatic cells.

Lymphocytes cultures were established from blood samples of 103 practically health individuals, who were informed about and agreed with the study. 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 4 h with colcemid). This procedure made it possible to analyze cells in the first post-radiation mitosis. Routine preparations were made and stained with 2% Giemsa solution. The analysis of painted chromosome preparations was carried out according to the conventional requirements to metaphase spreads [12]. The study was approved by Ethic Committee of IEPOR.

γ-Irradiation (^{60}Co) of PBL cultures was carried out at 1.0–3.0 Gy dose range and 1.0 Gy/min dose rate during different cell cycle stages (G_0 — at 0 h, G_1 — 24 h, S — 40 h i G_2 — 46 h of cell incubation). For G_2 treatment cultures of PBL were irradiated with dose of 1.5 Gy.

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 ±, 96*SD*. Radiosensitive cut-off point was also calculated as the 90th 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

Estimation of radiation-induced cytogenetic effects usually requires the data on spontaneous aberrations level. It is known to be not exactly fixed value since it can be influenced by patient age, modifications in culture incubation, effects of different mutagenic agents etc. Therefore the necessity to obtain own data on aberration spontaneous level is obvious. Total frequency of chromosome aberrations in the examined group (n = 103) was in the limits 2–7%, with mean 2.49 ± 0.14 aberrations per 100 metaphases. Chromatid deletions and isodeletions were the most frequently aberration types (89%). Chromatid-type/chromosome-type ratio was 4.8 : 1. Individual differences were formed by chromatid aberrations reference for spontaneous mutagenesis. Thus spontaneous levels obtained differed among donors and exceed the mean population value indicated in literature [13].

Our previous studies have shown that cytogenetic reaction of human chromosomes to radiation exposure changes during the cell cycle not only in dependence on its stage but also within the limits of each of them [14, 15]. Thus before the determination of IR of healthy donors the detailed study of PBL chromosomal radiosensitivity after irradiation in different cell cycle stages was carried out resulting in the obtaining of own calibration "stage-effect" curves. Main cytogenetic parameters: % of damaged cells, total number of aberrations, levels of chromatid and chromosome types aberrations, chromatid and isochromatid breaks, chromatid exchanges were examined taking into account irradiation and culture conditions.

This study revealed two picks in chromosomal radiosensitivity: at the late G_1- and G_2 -stages while S-stage was radioresistant. During cell cycle progression regular change of chromosome-type aberrations to chromatid one was observed. At the first half of cell cycle exchange aberrations prevailed, while fragments — at the second. Exchanges/fragments ratio in the first half of cycle equaled 2 then it gradually reduced due to the decrease of exchanges. At 30 h of cell cycle it was less than 1. Thus under the irradiation in G_0-, G_1- stages chromosome type aberrations prevail in the spectrum of radiation-induced damages; in G_2 — chromatid type and in S — both types. The highest level of fragments, namely chromatid breaks were observed after G_2-stage irradiation. Subsequently we registered next types of chromatid breaks, which objectively are recognized according the criterions (Fig. 1): fragments displaced along chromatid length; fragments displaced along chromatid axis; fragments inverted relatively to the axis. Chromatid interstitial deletions were also registered as chromatid fragments.

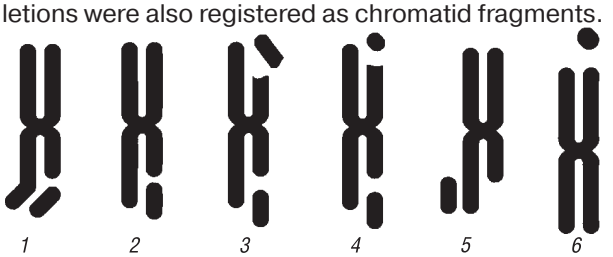

Fig. 1. Registered types of chromatid fragments

On the basis of the obtained "stage-effect" and "dose-effect" calibrating curves the scheme of cytogenetic examinations of healthy individuals was developed (Fig. 2). It assumes connection of G_2-assay principles and the statements of the classical radiation cytogenetics. According to them such factors as dose value, cell cycle stage, post irradiation conditions effect the estimation of quantitative and qualitative variations in cell radiation response [16].

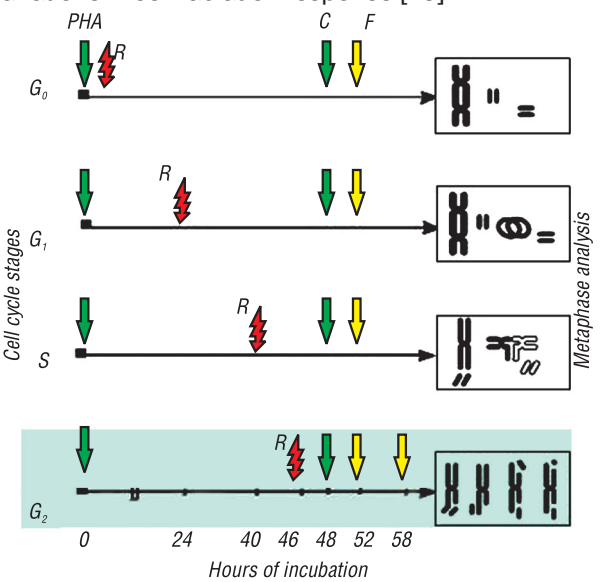

Fig. 2. The scheme of cytogenetic experiments on the assessment of individual radiosensitivity of healthy individuals (PHA — phytohaemagglutinin, R-γ-radiation, C — colcemid, F — fixation)

Detailed discussion of the data obtained with the help of cytogenetic examinations of healthy donors with the aim of the assessment of their IR is presented bellow.

– Dose of test irradiation. Linear dose dependence of fragments number after G_2 irradiation in wide dose range was obtained. We also determined the dose — 1,5 Gy which allows to obtain the values of mitotic index sufficient for metaphase number (mean scored number — 200 metaphases), the objective estimation of radiation-induced effects and registration of individual variations in karyotype sensitivity to test irradiation.

– Examination of test irradiation time in the limits of radiosensitive G_2 stage made it possible to reveal highest variations in IR values. Table 1 presents cytogenetic data obtained after γ-irradiation of PBL culture at 1.5 Gy in the dependence from the time of cell incu-

bation during G_2 stage (42. 44 and 46 h). The highest levels of aberrant lymphocytes (31.0 ± 0.9), total number of aberrations (31.0 ± 0.9), chromatid aberrations (38.0 ± 1.6) and chromatid breaks (32.0 ± 1.6) were registered after irradiation at 46 h of cell incubation, at the end of G_2 stage. This term is proposed for test irradiation as allows observing the highest differences in chromosomal radiosensitivity.

Table 1. Frequencies of chromosomal aberrations in human lymphocytes exposed to 1.5 Gy γ-rays at G_2-stage of cell cycle

Irradiation time (h)	Abnormal cells, %	Chromosomal aberrations/100 cells	Chromatid aberrations/100 cells	Breaks/ 100 cells
42	18.5 ± 1.9	22.0 ± 2.1	21.2 ± 2.3	20.0 ± 1.2
44	26.9 ± 2.3	33.0 ± 3.1	29.0 ± 2.5	24.0 ± 2.3
46	31.0 ± 0.9	40.6 ± 1.4	38.0 ± 1.6	32.0 ± 1.6

Radiation-induced cell cycle delay. Fixation of cells irradiated in G_2 stage in two terms — 52 h and 58 h after the beginning of cultivation was carried out (Fig. 3). It was revealed that cytogenetic data of intercellular aberrations distribution obtained after G_2 test irradiation and 52 h cultivation fitted Poisson distribution. This fact testifies that the effect of radiation-induced cell cycle delay plays insignificant role and cell population is relatively homogeneous. After fixation at 58 h deviations from the theoretical distribution was observed, which indicated the heterogeneity of cell population (mixture of first and second post radiation mitosis) and made these data less informative [16, 17].

– Observation of standard conditions of cell cultivating, irradiation and fixation is obligatory requirement for the analysis and comparison of the obtained cytogenetic parameters and objective IR estimation on their basis. These optimal laboratory conditions are indicated in the scheme (see Fig. 2, bottom line).

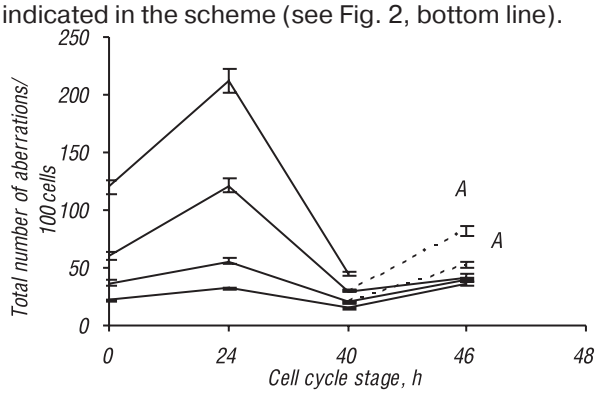

Fig. 3. Kinetics of chromosomal aberrations frequencies in human lymphocytes exposed to γ-rays during cell cycle. Lines A — fixation on 58 h of cultivation, rest — on 52 h

The developed scheme of the assessment of IR of healthy individuals on the basis of G_2-assay was approved during cytogenetic examination of healthy Kyiv habitants (n = 103). Analysis of cytogenetic parameters induced by G_2 irradiation at 1.5 Gy dose revealed their high interindividual variability (Table 2). The highest differences were registered for chromatid type aberrations (*CV* = 42.1%) with the chromatid break predominance in the spectrum (84% from the total aberrations number, up to 95% for individual donors, *CV* = 37.5%). This made it possible to consider chromatid breaks to be the specific marker of chromosomal radiosensitivity after G_2 irradiation.

Table 2. G_2 radiosensitivity of human lymphocytes derived from healthy individuals (n = 103) and exposed to 1.5 Gy γ-rays

Cytogenetic parameter	Mean number M ± SE	SD	CV, %
Abnormal cells, %	37.9 ± 1.08	9	29.0
Total aberrations /100 metaphases	40.6 ± 1.8	14	34.5
Chromatid breaks /100 metaphases	32.0 ± 1.6	12	37.5

To indicate the cut-off points of individual variations of the obtained IR cytogenetic parameters we used two approaches: calculation of 90% percentile values of their distributions and analysis of its character. In our case the value 90% cut-off point was 64 aberrations/100 metaphases that indicated 12% individuals with increased chromosomal radiosensitivity.

Analysis of the obtained cytogenetic parameters' distributions in the referent group on the basis of variation statistics showed that they did not fitted normal distribution (Shapiro-Wilki test, w = 0.86; $p = 0.05$ for chromatid breaks). Two picks in aberration frequencies were observed (Fig. 4). Fitting the obtained distribution to the sum of two normal ones ($R^2 = 0.99$) made it possible to indicate mean values of cytogenetic parameters for two subgroups: individuals with normal and high chromosomal sensitivity to radiation (Table 3). Calculation of 95% confidential interval of their variation as M ± *2 SD* makes it possible to know high cut-off value in group of donors with normal IR: 63.1 aberrations/100 metaphases, which is practically coincides with 90-percentile values (64 aberrations/100 metaphases, 12% radiosensitive individuals). Observed bimodality in the distribution of IR cytogenetic parameters induced by the test irradiation in G_2 stage of cell cycle testifies for the heterogeneity of the referent group and possible existence of two populations among healthy individuals – with normal and enhanced chromosomal radiosensitivity, which is determined genetically. It is suggested that such predisposition to the elevated G_2 radiosensitivity is a consequence of the inherited defects in the efficiency of DNA repair system which predispose to cancer [5, 18, 19].

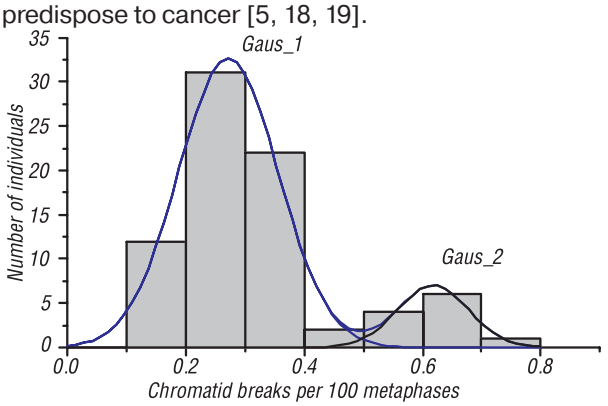

Fig. 4. G_2 chromosomal radiosensitivity of healthy donors (n = 103) at 1.5 Gy γ-irradiation of lymphocytes

Table 3. Summary of cytogenetic parameters induced by 1.5 Gy test irradaition of lymphocytes of healthy donors from two groups – normal (I) and elevated (II) G_2 chromosomal radiosensitivity

Cytogenetic parameter/100 metaphases	Group of donors	Mean number M ± SE	SD	CV, %
Total number of chromosomal aberrations	I	37.7 ± 1.8	12.7	33.6
	II	74 ± 2.2	8.9	12.0
Chromatid aberrations	I	33.7 ± 1.1	12	36.3
	II	67 ± 2.4	7	10.4
Chromatid breaks	I	27 ± 1.4	10	37
	II	61 ± 1.8	8	13

Taking into account obtained results, cytogenetic examination of healthy individuals on the base of G_2-assay has its perspectives in the formation of groups with increased risk of cancer developing and its primary prophylactics among healthy population.

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ПОВЫШЕННАЯ ИНДИВИДУАЛЬНАЯ РАДИОЧУВСТВИТЕЛЬНОСТЬ ХРОМОСОМ ЛИМФОЦИТОВ ЧЕЛОВЕКА КАК ПОКАЗАТЕЛЬ РИСКА ЗЛОКАЧЕСТВЕННЫХ НОВООБРАЗОВАНИЙ

Цель: **оценка радиочувствительности здоровых лиц на уровне хромосом лимфоцитов и определение индивидуумов с повышенным риском радиоиндуцированных новообразований.** ***Методы:*** **цитогенетическое исследование радиационно индуцированных повреждений в лимфоцитах здоровых доноров (n = 103) проведено на основе G_2-теста. Использована тест-система лимфоцитов периферической крови с последующим метафазным анализом.** ***Результаты:*** **на основе построения калибровочных кривых "стадия-эффект", "доза-эффект" разработана схема цитогенетического обследования условно здоровых лиц. Анализ величин цитогенетических показателей, полученных при облучении в G_2-периоде клеточного цикла в дозе 1,5 Гр, выявил их значительную вариабельность. Наибольшая вариабельность наблюдалась для аберраций хроматидного типа (*CV* = 42,1%), в спектре которых преобладали одиночные хроматидные разрывы (*CV* = 37,5%). Статистический анализ распределений индивидуальных значений полученных показателей позволил выявить 12% лиц с повышенной радиационной чувствительностью хромосом.** ***Выводы:*** **цитогенетическая оценка индивидуальной радиочувствительности на хромосомном уровне, основанная на G_2-тесте, имеет перспективы применения при формировании групп повышенного риска радиоиндуцированных злокачественных новообразований и их первичной профилактики среди населения.**

Ключевые слова: **радиочувствительность, G_2-тест, радиоиндуцированные новообразования.**