ORIGINAL ARTICLE

Calibration Curve for Dicentric Chromosomes Induced in Human Blood Lymphocytes Exposed to Gamma Rays at a Dose Rate of 12.5 mGy/s

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ABSTRACT

To develop a calibration curve for induction of dicentric chromosomes by radiation, we have used a 60Co gamma-ray source with dose rate of 12.5 mGy/s. Whole blood from 15 healthy donors was collected. Whole blood from each donor was divided equally into 8 parts for exposing to supposedly physical doses 0, 0.30, 0.50, 1.00, 1.50, 2.00, 3.00 and 4.00 Gy for a independent calibration curve. Whole blood from 15 donors was used to calibrate dose – effect and statistical for general calibration curve. Using Poisson test (U-test) for the distribution of dicentric chromosomes in the metaphases to determine the uniformity of the radiation field. The average from 15 independent calibration curves of linear correlated coefficient was determined to be *r* (y, d) = 0.5136 ± 0.0038. The model equation derived is $y = aD + bD^2 + C$. The calibration equation of dose-effect was $y = 1.01D + 4.43D^2 + 0.56$.

Key words: Exposed dose point, gamma rays, general curve, independent curve, uniform radiation field

Introduction

The tools of biodosimetry for monitoring individuals exposed to environmental, occupational, clinical, or accidental radiation are background dataset and calibration dose–effect dataset. These datasets need to be created by each biodosimetry laboratory to strengthen their preparedness in response to radiation emergencies. In addition, calibration or dose–response curve can be generated with good regression coefficients but are characterized by linear energy transfer (LET) and dose rate of radiation source. Therefore, choosing an appropriate calibration dose–effect curve close to radiation source to which victim exposed in terms of LET and dose rate is required. The calibration of dose–effect for ^{60}Co gamma-ray source with dose rate of 12.5 mGy/s was chosen for this study.

Materials and Methods

Blood was drawn from 15 healthy donors (22–45 years of age) and each blood sample was divided into eight parts for exposing it to radiation doses 0, 0.3, 0.5, 1.0, 1.5, 2.0, 3.0, or 4.0 Gy. A ^{60}Co gamma ray source (gamma cell – Isscledavatel, Russia) was used to irradiate the blood at the dose rate of 12.5 mGy/s.

The component of radiations and the component of radiation dose were also determined by physical dosimetry method and chemical method (Fricke) at Nuclear Research Institute, Vietnam. The absorbed dose values were counted for each exposed times of all supposed dose points, mean that 105 exposed dose values and 15 controls had presented for this calibration. Following radiation exposure, lymphocytes were cultured in RPMI 16040 medium supplemented with phytohemagglutinin and fetal calf serum (Sigma), incubated for 48 h/37°C. Colcemid was added 2 h before harvesting to prepare metaphase spreads. Dicentric chromosome aberration analysis relied on centromere, number of fragments, and according to classification of chromosome aberration in the first cell cycle.[1-6] Data generated were subjected to *t*-test for comparisons. In addition, Poisson test was used to estimate dicentric chromosome distributions induced in exposed lymphocytes. The hypothesis of Poisson distribution was done according to the standard of Chi-square. The samples

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are exposed in uniform radiation field if Chi-square value fits hypothesis value.[2,7,8] The correlation coefficient r (y, d) was used to find model equation, and recurrent equation was solved to find recurrent coefficients for independent curves, statistical and presenting general calibration dose–effect curve.[6-10]

Results and Discussion

The dicentric data from 15 independent samples were used to generate a composite calibration curve. In this case, we notice that there are individual variations among the different samples. However, 120 data couples on related dose–dicentric were used to find Poisson distribution, linear correlated coefficients, model equation, experimental regressive coefficients, and general calibration equation of dose–effect curve.

Testing of Poisson distribution

Poisson distribution of dicentric chromosomes among metaphases was used to check the uniformity of the radiation field. A total of 548 dicentrics distributed in 758 cells with the number dicentric in each cell are presented in Table 1.

Hypothesis of Poisson distribution was tested according to the standard of Chi-square with formula $\chi^2 = \sum_i^k \chi_i^2 = \sum_i^k ([m_i - np_i]^2/np_i)$ (i:i = 0, 1, 2, 3 ... is natural number showed number dicentric in a cell; m_i is the number of cells have i dicentric; n is the total cells analyzed; p_i is the theory numerical value of Poisson, p_i = N'x/N = $e^{-\lambda}$. $\lambda^x/x!$; λ: Average frequency of dicentric per cell, $\lambda = \sum m_i/n$). The parameters of Chi square were $\lambda = \sum mi/\sum n = 548/758 = 0.72$; $e^{-\lambda} = 0.48$; $\sum_i \chi^2 = 1.21 + 0.53 + 1.47 + 0.06 + 0.33 = 3.60$. Consulting Chi-square table with k = 5, $\alpha = 0.05$ had χ^2_{k-1} (α) = 9.19. The data presented $\sum \chi_i^2 < \chi^2_{k-1}$ (α) which essentially means that dicentrics induced in the cells fited in Poisson distribution. This result ensured the reliability of the samples exposed in the uniform radiation field.

Finding model equation of dose–effect response

The correlation coefficient, r (y, d) = 0.514 ± 0.004, illustrates that there was a average linear correlation between doses and dicentric frequencies, but diagram [Figure 1] of correlation between dose and frequencies of dicentric shows a parabolic form for all of the 15 combinations. The r (y, d) reflected exactly the effect of low LET radiation. The general experimental equation had the form of quadratic equation: $y = \alpha D + \beta D^2 + C$ [Table 2].

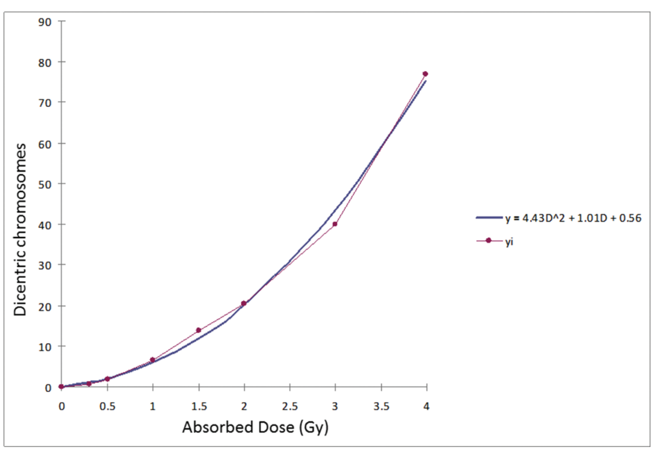

Figure 1: The dose–effect calibration curve for dicentric chromosomes in human blood lymphocytes (red - experimental data, blue - graph of calibration equation)

Counting experimental recurrent coefficients of model equation $y = \alpha D + \beta D^2 + C$

Finding the experimental recurrent coefficients α, β, and X of quadratic equation: $y = \alpha D + \beta D^2 + C$ of the independent curves was conducted by solving of the set of three equations:

$$cn + \alpha \Sigma D_i + \beta \Sigma D_i^2 = \Sigma y_i \quad (1)$$

$$c\Sigma D_i + \alpha \Sigma D_i^2 + \beta \Sigma D_i^3 = \Sigma y_i D_i \quad (2)$$

$$c\Sigma D_i^2 + a\Sigma D_i^3 + \beta \Sigma D_i^4 = \Sigma y_i D_i^2 \quad (3)$$

In these equations, D_i indicates the absorbed doses that used for exposing blood samples (eight doses) and y_i is induced dicentric frequencies to D_i. Using experimental data of dose–effect and using replaced method for solving of the set of three equations above produced, the recurrent values of α, β, and C are shown in Table 3.

Table 3 presents the values α, β, and C of 15 independent curves, the averages of these values were $\alpha = 1.01 \pm 0.93$; $\beta = 4.43 \pm 0.30$; C = 0.56 ± 0.39.

The general experimental recurrent coefficients were $\alpha = 1.01 \pm 0.93$; $\beta = 4.43 \pm 0.30$; C = 0.56 ± 0.39 ($\alpha = 10^{-2} \times Gy^{-1}$;

Table 1: Distribution of dicentric per cell in 10 random samples of 15 independent curves exposed to gamma radiation

Number dicentrics in a cell	Number of cells scored	χ^2
0	389	1.21
1	254	0.53
2	108	1.47
3	22	0.06
4	3	0.33

Table 2: The results of linear correlative coefficients for 15 independent curves

IC	r (y, d)
1	0.509
2	0.511
3	0.515
4	0.511
5	0.512
6	0.516
7	0.514
8	0.508
9	0.514
10	0.517
11	0.516
12	0.516
13	0.520
14	0.519
15	0.518

r (y, d) = 0.514±0.004. IC: Independent curves

Table 3: The results of the experimental recurrent coefficients of 15 independent curves

Coefficient	The IC														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
α	0.29	0.51	0.29	1.53	1.00	2.29	2.85	0.28	2.16	0.22	1.04	2.03	0.05	0.15	0.45
β	3.98	4.80	4.86	4.45	4.02	4.17	3.98	4.41	4.24	4.70	4.59	4.43	4.49	4.70	4.70
C	1.10	0.99	1.03	0.20	0.77	0.52	0.01	1.31	0.20	0.42	0.28	0.14	0.46	0.49	0.48

IC: Independent curves

$\beta = 10^{-2}$ Gy^{-2}). The general experimental recurrent equation was $y = 1.01D + 4.43D^2 + 0.56$.

The dose–effect calibration curve is shown in Figure 1 (red - experimental data, blue - graph of the general calibration equation).

The data from our study indicated that the samples were exposed in the uniform radiation field. Poisson distribution (U-test) of dicentric chromosomes among metaphases was a parameter that used to check the uniformity of the radiation field.[2,6,7,9] Consulting Chi-square table with $k = 5$, $\alpha = 0.05$ had $\chi^2_{k-1}(\alpha) = 9.19$. The data presented $\sum\chi_i^2 < \chi^2_{k-1}(\alpha)$, it means that induced dicentric distribution in the irradiation cells fitted well with Poisson distribution. This result ensured the reliability of the samples exposed in the uniform radiation field.

Dose–effect relationship followed to the model equation with linear correlative coefficient. The correlation coefficient r (y, d) $= 0.514 \pm 0.004$ did not fit linear correlation between doses and observed dicentric frequencies, but fitted reasonably well with an exponent correlation. A weak correlation coefficient r (y, d) $= 0.514 \pm 0.004$ fitted with a dose–effect distribution $y = \alpha D + \beta D^2 + C$ that was in accordance with low LET radiation in observed source. Our result showed the suitability of the basic principles of radiation effects, such as dependence on LET in line with earlier reports.[2,4,6,11-16]

The dependence of model equation on the LET as well as the dependence of the coefficient's rate α/β of the calibration equation of dose–effect on dose rate of radiation sources have been observed in earlier publications.[10-16] Solving of experimental recurrent equation $y = \alpha D + \beta D^2 + C$ showed the experimental recurrent coefficients $\alpha = 1.01 \pm 0.93$ (10^{-2} Gy^{-1}), $\beta = 4.43 \pm 0.30$ (10^{-2} Gy^{-2}), $C = 0.56 \pm 0.39$ (10^{-2}), $\alpha/\beta = 0.228$ and calibration dose–effect $y = 1.01D + 4.43D^2 + 0.56$. Our result showed the suitability of the basic principles of radiation effects, such as dependence on energy, LET, and dose rate.

Conclusion

Calibration of dose-effect was conducted for ^{60}Co gamma rays with dose rate of 12.5 mGy/s. The investigated data of 15 indipendent calibration curves presented that distribution of dicentric chromosome among metaphases of exposed cells was fitted a Poisson distribution with p = 95%, this evidence showed irradiation field was uniform radiation field.

The linear related coefficient r (y, d) $= 0.514 \pm 0.004$, equation model fitted to $y = \alpha D + \beta D^2 + C$. The experimental recurrent coefficients were determined $\alpha = 1.01 \pm 0.93$ (10^{-2} Gy^{-1}); $\beta = 4.43 \pm 0.30$ (10-2 Gy^{-2}) and $C = 0.56 \pm 0.39$ and calibration dose-effect was presented Y = 1.01D + 4.43D2 + 0.56.

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Conflicts of interest

There are no conflicts of interest.

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