Biological Dosimetry of Co-60 Gamma Irradiation

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ABSTRACT

Development of radiation technology has resulted in increasing numbers of people working with it. Therefore it has increasingly been important to monitor the radiation in order to ensure public safety. Physical dosimetry plays an important role in monitoring. But a need arise for biological dosimetry where physical dosimetry is absent or its presence is insufficient.

In this study Co-60 gamma radiation dose-response curves for chromosome aberrations were determined for use as controls in biological dosimetry. Peripheral blood that were taken from healthy individuals not working with radiation were irradiated at different radiation doses. The relationship between unstable chromosome aberrations in metaphaseblocked cells and radiation dose were drawn by using the linear-quadratic (LQ) formula.

The absorbed radiation doses of the test group consisting of five people that had been working with Co-60 teletherapy machines were estimated using the LQ parameters of control dose-response curves in the Q_{dr} method. Estimated radiation doses were below the permissible radiation dose-limits for four workers, but one worker's estimated dose was higher than these limits.

Key Words: Biological dosimetry, Co-60 y-irradiation, Chromosome aberrations.

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INTRODUCTION

Undesired irradiation may occur in the case of radiation accidents in places where the radiation in use or irradiation may result from a geographical locations naturally. Absorbed radiation doses from all sources should be kept below the permissible dose levels for both public and radiation workers[1]. Physical dosimeters may not be available for all these circumstances to monitor radiation. Therefore radiation dose estimations from blood lymphocytes provide a valuable tool in assessing effects of radiation on irradiated persons[2,3,4,5,6].

Discrete features of being mostly in non-dividing phase (G_0-phase) and thus having a long life, and being sensitive to radiation and easy handling make lymphocytes a very useful compo-

nent in biological dosimetry. Nevertheless past exposures and non-uniform irradiations produce difficulties in estimating absorbed radiation doses[2,7]. The Q_{dr} method of Sasaki [8] relating aberrations (dicentrics and centric rings) only in damaged cells to a radiation dose overcomes the problems of dependency on time after exposure and inhomogeneous irradiation. The background frequencies of chromosome aberrations in different populations vary depending on the variations in biological diversity, geographical situation, atmospheric pollution, the extent of environmental clastogenic chemicals, or the use of medical drugs alone or in combinations [9,10]. In addition yields of chromosome aberrations following different types of radiations differ. Therefore each biological dosimetry laboratory should establish its own control dose-response curves for any different LET radiations available. We have established in this paper control doseresponse curves for Co-60 gamma radiation and applied these curves to estimate absorbed radiation doses of five people that had been working with Co-60.

MATERIALS and METHODS

Control Dose-response Curves

Peripheral blood was taken from 2 male and one female non-smoker healthy donors with no radiation working history. Their age differed between 28-48 years at the time of blood sampling. Microculture method of Moorhead et. al.[11] was used with small modifications. For each donor, after 4 hours of irradiation 0.5 ml of irradiated whole blood was added in culture containing 4 ml of RPMI-1640 with glutamine (Sigma) supplemented with lml newborn calf serum, 100 g/ml streptomycin, 100 IU/ml penicillin and 15 g/ml phytohemagglutinm, and incubated at 37° for 45 h. After adding 1 g/ml colcemid solution, cells were incubated for another 3 h. Fixation, staining and chromosome preparations were performed according to standard procedures[2] with minor modifications.

In proliferating cells 50% of dicentric chromosome aberrations are lost in the first division following irradiation[12], leading to underestimation of radiation doses. Therefore radiation dose estimations should mainly be based on the first cycle metaphases. A ratio of M_2 metaphases to M_1 metaphases should not be more than 10% in order to base the radiation dose estimations mainly on first division metaphases[2]. Total culturing time of lymphocytes in our study gave M_2/M_1 ratio of 7.8% after fluorescence plus Giemsa staining[13], indicating that chromosome aberration analyses were carried exclusively on first cycle metaphases.

Irradiation

Irradiation was performed by using Alcyon II Co-60 teletherapy machine at 42.5 cGy/min. Heparinized tubes containing 4.5 ml of donor's blood were irradiated with bolus homogeneously at 10 different doses between 0.10-5.00 Gy at 37° and one left for control.

Scoring Chromosome Aberrations

Unstable chromosome aberrations of asymmetrical exchange types were scored. Control dose-response curves were established from dicentric and excess acentrics yields at different radiation doses. Homogeneous low LET radiations produce random ionizations in cell leading to random distribution of chromosome aberrations in low frequencies especially at low doses and this follows Poisson distribution[3,14]. Overdispersion is observed in non-uniform aberrations. Magnitude of overdispersion is related to the heterogeneity of irradiation[15]. In order to test the homogeneity of irradiation, the dispersion index (s2/y; the ratio of variance to dicentric yield) was calculated at each radiation dose. If the dispersion index equates to 1 it can be presumed that dicentrics are distributed according to Poisson. In addition U-test [16,17]was used to acquire a statistical evidence of whether the ratio s2/y differs significantly from 1. Magnitude of test quantity U which approximates to a unit normal deviate and which is between the values of -1.96 and 1.96 relates to Poisson distribution.

A weighted least square regression analysis (In Plot; GraphPad Software Inc.) was used to fit the dicentric data to the linear-quadratic model, $y = aD + ßD^2$, by minimizing residual sum of squares (weights were chosen as a Poisson estimate).

RESULTS

Total of 12022 cells were analyzed. In scoring aberrations from metaphase cells through the microscope, chromosome pieces less than 46 were left out of an analysis. Figure 1 shows dicentric, tetracentric and acentric chromosome aberrations after 5.00 Gy irradiation.

Unstable chromosome aberrations following different radiation doses were recorded (Table 1). The acentric fragments associated with dicentrics, tricentrics, tetracentrics or rings which were direct consequence of irradiation were not included in the number of excess acentrics. Increasing radiation dose resulted in an increasing number of aberrations.

Higher yields of dicentric and excess acentric aberrations were apparent compared to other aberrations. They have been used to make control dose-response curves in estimating absorbed radiation dose. Intercellular distribution of dicentric chromosomes at each radiation dose is given in Table 2. The number of dicentrics were increased with increasing radiation dose. Yield of dicentrics at 0.00 Gy dose which relates to the natural background was $0.55x10^{-3}$. Increases in dose resulted higher numbers of dicentrics distribution in cells. In order to test the homogeneity of irradiation the dispersion index (s2/y) and the magnitude of statistical test quantity U were also given in Table 2. The dispersion index centers around 1 nearly for each radiation. Calculation of U-test showed that each distribution is Poisson as the values ranged between -1.96 and 1.96. Thus irradiations were homogeneous.

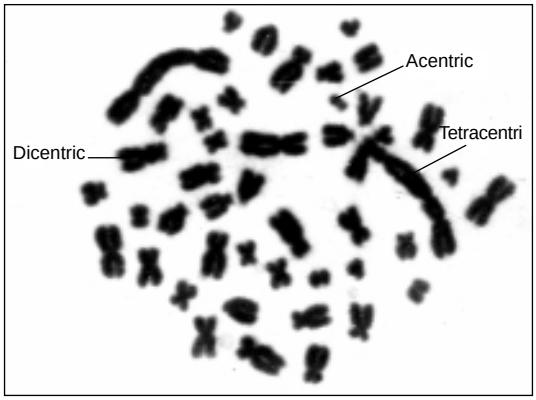

Figure 1. Metaphase chromosomes containing dicentric, tetracentric and acentric chromosome aberrations following 5.00 Gy irradiation.

Control dose-response curve of the yield of dicentric aberrations as a function of radiation dose was drawn in Figure 2. Dose-effect relationship was expressed with the linearquadratic model, $y = aD + BD^2$. In this linear-quadratic equation a represents linear component where chromosome

Table 1. Distribution of chromosome aberrations for different doses of Co-60 gamma radiation

Dose (Gy)	Metaphase scored	Number of dicentrics	Number of tricentrics	Number of tetracentrics	Number of centric rings	Number of excess acentrics
0.00	1828	1				1
0.10	1743	4				13
0.25	1269	9				13
0.50	1514	28				26
0.75	858	25			3	34
1.00	1485	99	1		1	88
1.50	1289	225			7	126
2.00	979	223			12	139
3.00	324	158	1		2	110
4.00	375	351	6		12	187
5.00	358	554	10	1	41	327

aberrations are the result of single-track events and it is mostly responsible from aberrations at low doses., ß represents quadratic component where chromosome aberrations are the result of two-track events and it is mostly responsible from aberrations at high doses. The values of a and ß with their standard errors were 0.34 x $l0^{-2}$ ± $7.29x10^{-3}$ and $6.05x10^{-2}$ ± $2.82x10^{-3}$ respectively. The a/ß ratio of dicentric yield in this study was 0.06 Gy representing the dose at which both track events are responsible from the aberrations equally. This shows that the contribution of ß-component is evident even at low doses of irradiation.

Intercellular distribution of acentric fragments not associated with dicentrics, tricentrics, tetracentrics or rings at different radiation doses is given in Table 3. The number of excess acentrics were increased with increasing radiation dose. The background levels of excess acentrics was $0.88x10^{-3}$. At high doses of radiation, higher numbers of acentric distribution in cells were observed. When the Poisson statistics was applied it was shown that the dispersion index (s2/y) was not around l for some of the radiation dose points (Table 3). U-test also confirmed this by showing the values at 1.00 Gy, 2.00 Gy, 4.00 Gy and 5.00 Gy deviating out of the significance range for Poisson distribution.

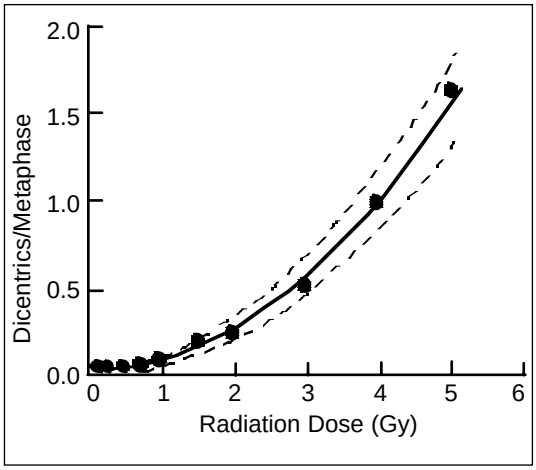

Figure 2. Co-60 gamma radiation induced linear-quadratic dose response curve for dicentric chromosomes. Dotted lines represent 95% confidence intervals.

Dose-response curve of the excess acentric yield was fitted to the linear-quadratic model in Figure 3. The values of a and, ß with their standard errors were 2.36 x 10^{-2} ± 0.68 x 10^{-2} and 2.89 x 10^{-2} ± 0.23 x 10^{-2} respectively and the value of a/ß ratio was 0.82 Gy. Both single-track and two-track events are responsible from the aberrations at low doses.

In order to apply our control dose-response

Table 2. Intercellular distribution of dicentric chromosomes for different doses of Co-60 gamma radiation

Dose	Metaphases	Number of	Distribution							s2/y	U
(Gy)	scored	dicentrics	0	1	2	3	4	5	6		
0.00	1828	1	1827	1						1.00	0.00
0.10	1743	4	1739	4						1.00	-0.06
0.25	1269	9	1260	9						0.99	-0.17
0.50	1514	28	1486	28						0 98	-0.50
0.75	858	25	834	23	1					1.05	1.10
.00	1485	101	1388	93	4					1.01	0.33
I.50	1289	225	1080	193	16					0.97	-0.80
2.00	979	223	785	169	21	4				1.07	1.54
3.00	324	160	193	105	23	3				0.91	-1.16
4.00	375	363	143	136	69	19	8			0.99	-0.09
5.00	358	577	63	119	104	48	15	8	1	0.89	-1.45

Table 3. Intercellular distribution of excess acentrics for different doses of Co-60 gamma radiation

Dose (Gy)	Metaphases secored	Number of excess acentrics	Distribution							s2/y	U
			0	1	2	3	4	5	6		
0.00	1828	1	1827	1						1.00	0.00
0.10	1743	13	1730	l3						0.99	-0.21
0.25	1269	13	1256	13						0.99	-0.25
0.50	1514	26	1489	24	1					1.06	1.70
0.75	858	34	825	32	1					1.02	0.43
1.00	1485	88	1405	72	8					1.12	3.38
1.50	1289	126	1170		112	7				1.01	0.36
2.00	979	139	855	110	13	1				1.09	1.98
3.00	324	110	230	80	12	2				0.99	-0.12
4.00	375	187	244	87	33	10	1			1.24	3.33
5.00	358	327	165	105	56	22	7	2	1	1.31	4.11

curves to estimate an absorbed radiation dose, 5 people working with Co-60 gamma radiation for a reasonably long time of 2-24 years of range were chosen. Dicentrics and accentric fragments from their peripheral blood lymphocytes were scored (Table 4). For only the number 4 worker one centric ring was observed and not included in the Table. Radiation dose estimations were performed by using the Q_{dr} method of Sasaki[15]. The Q_{dr} value is the ratio of the number of dicentrics and rings to the cells containing dicentrics, rings and acentric fragments;

$Q_{dr} = Y_{dr}/1\text{-}exp\text{-}(Y_{dr} + Y_{ace})$, Y_{dr} is the dose response relationship for dicentrics and centric rings, and Yace is the dose response relationship for excess acentrics. The dose response relationship for dicentrics (Y_d) was used instead of Y_{dr}. Calculations of Q_{dr} are shown in Table 4.

DISCUSSION

A method of scoring unstable chromosome aberrations which defines the morphological cytogenetic changes easily in biological dosimetry is a worthwhile over to scoring stable aberrations[4,5]. Evaluating stable aberrations requires chromosome banding techniques which are expensive. These type of aberrations are not lost through cell divisions whereas unstable aberrations are. Therefore past exposures can be scored in cells where the aberrations persist throughout the life span of lymphocytes.

Loosing the unstable aberrations as cells divide may result in underestimation of radiation doses. This problem is overcome by several methods. Of these the Q_{dr} method by Sasaki[8] which takes only the damaged cells into the consideration provides an invariable tool in estimations excluding the possible conflicts that can be raised consequent to past exposures, or even partial

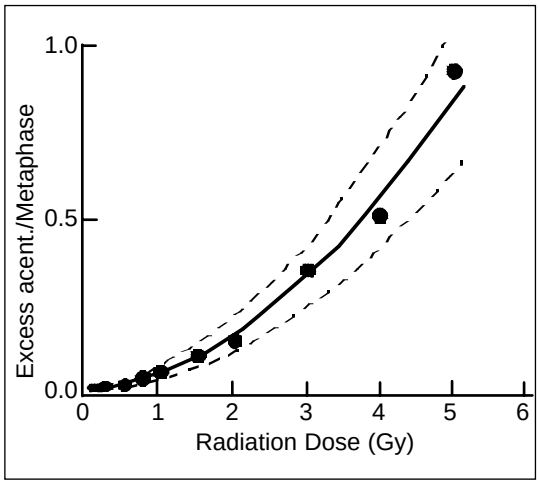

Figure 3. Linear-quadratic dose-response curve for excess acentric chromosomes. Dotted lines represent 95% confidence intervals.

Table 4. Analysis of chromosome aberrations in medical-radiation workers

Workers	Sex	Age	Smoker (S) or non-smoker (NS)	Metaphases scored	Number of dicentrics	Number of excess acentrics	Estimated dose (mGy)
1*	K	36	NS	724	-	2	< 0.1
2*	K	48	NS	904	-	2	< 0.1
3*	E	28	NS	814	-	5	< 0.1
4**	K	45	S	1851	-	13	5.8
5**	E	38	S	473	3	4	583

*; Radiation physicist

**; Radiation technician

body or non-uniform exposures. Studies [18] have shown that scoring either unstable or stable aberrations give similar results of dose estimations.

We have established in this paper a control dose-response curves of dicentric aberations and excess acentrics for Co-60 gamma irradiation. 10 different radiation doses were used from 0.10 Gy to 5.00 Gy. There are 4 dose points at low doses between control and 1.00 Gy dose range at which most of the possible radiation accidents occur[19]. The yields of both dicentrics (Table 2) and acentrics (Table 3) were increased with increasing radiation dose. Less amounts of metaphases were observed as radiation increases which was due to the interphase death of lymphocytes bringing less cells for metaphase analysis. Dicentric yield at 0.00 Gy dose which relates to the natural background was $0.55x10^{-3}$. Homogeneity of irradiation was confirmed in this study by showing the distribution of dicentrics following different radiation doses were Poisson (Table 2). Deviations from the Poisson for some of the dose points were observed in the distribution of excess acentrics 13 (Table 3). Formation of excess acentrics are not specific to radiation as they may occur as a result of an interaction with some other clastogenic agents. Therefore this type of aberrations were not used in radiation dose estimations alone. We have used the LQ parameters of the dose-response curve in calculating the Q_{dr} equation.

The relationships between chromosome aberrations and radiation (Figures 3 and 4) were best expressed with the linear quadratic equation. The linear-quadratic parameters, a and, ß giving the relationship between the yield of dicentric aberrations and radiation were 0.0034 and 0.0605 respectively. These values are comparable with the literature[14,18]. In applying the Q_{dr} method the ratio of the number of dicentrics + rings to the damaged cells containing dicentrics, rings and/or acentric fragments is related to the LQ parameters of the control dose-response curves in the method.

Radiation dose estimations by using the Q_{dr} equation for medical radiation workers (3 female and 2 male) are given in Table 4. Their employment history were between 2-24 years. One centric ring was observed in only the number 4 worker and no dicentrics were observed in the first, second and third workers. There were variations in the numbers of dicentrics and excess acentrics. The estimated doses were less than 0.1 mGy which is much below the permissible dose levels for 3 workers. The doses were 5,8 mGy and 583 mGy for the number fourth and the number fifth workers respectively. 5,8 mGy with 232 mGy of the upper level of 95% significance interval stays below 400 mGy which is the recommended sum of doses over total employment time[18,20]. But absorbed radiation dose estimation for the fifth worker (with 1371 mGy of the upper 95% significance level) give a sign of an over exposure according to the permissible dose levels.

In conclusion we have established the control dose-response curves of chromosome aberrati-

ons for Co-60 gamma irradiation in our biological dosimetry laboratory. This will enable us to estimate a magnitude of an absorbed radiation dose in any overexposed individual and to discuss the results with an other investigating laboratories for the benefit of a person.

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