ORIGINAL ARTICLE

Strong Correlation among Three Biodosimetry Techniques Following Exposures to Ionizing Radiation

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ABSTRACT

Three *in vitro* dose calibration curves for biodosimetry such as dicentric chromosome assay, fluorescence *in situ* hybridization (FISH) assay for translocation, and micronuclei (MNs) in binucleated cell assay were established after exposure to ionizing radiation. Peripheral blood lymphocyte samples obtained from healthy donors were irradiated with ^{60}Co source at a dose rate of 0.5 Gy/min to doses of 0.1–6 Gy. The results from three *in vitro* dose calibration curves for biodosimetry were analyzed to understand the relationship among biodosimetry assay techniques. Our comparison demonstrates that there is a very strong positive correlation among the dicentric assay, FISH, and MNs analysis, and these three biodosimetry assays strongly support the *in vitro* dose reconstruction and the emergency preparedness of public or occupational radiation overexposure.

Key words: Dicentric assay, fluorescence *in situ* hybridization, ionizing radiation, micronucleus assay, translocations

Introduction

Dose estimations of individuals occupationally exposed to ionizing radiation are currently carried out by the evaluation of personal dosimeters, for example, film badges, or by activity monitoring of the surroundings and subsequent calculations of the exposure. In the case of uncontrolled exposures, for example, accidental whole- or partial-body exposures, the exposed doses can be overestimated than the annual dose limit. In such situations, personal dosimeter readings are often imprecise or not available at all and the retrospective estimation of the dose will be necessary. Detailed knowledge of the doses received by individual provides the basis for follow-up examination and their further medical treatment. This will substantially reduce the possibility of further consequences. Dicentric chromosome (DC) assay, in biological dosimetry by the determination of the rate of unstable chromosome aberrations in peripheral blood lymphocytes, is commonly considered as "gold standard technique" of radiation when blood samples can be collected in 2 months after the exposure and are adopted at all reference biodosimetry laboratories.[1-4] Cytokinesis-block micronucleus (CBMN) assay is an additional assay used for radiation biodosimetry.[4,5] Recent reports from various laboratories have demonstrated its potential applicability for dose assessment. CBMN assay is now being considered as a very attractive tool for triage biodosimetry because of its advantages such as simplicity of scoring, accuracy, and most importantly, the easiness of automation using microscopy and flow cytometry.[5-12] Fluorescence *in situ* hybridization (FISH) is commonly used for retrospective dose estimations containing acute and chronic exposures. Consequently, this assay is being considered as the most suitable one for detecting occupational exposures.[3,4] The information obtained from these techniques may help to perform triage in radiation/nuclear mass casualty events (i.e., explosion of a nuclear power plant and terrorist attack with dirty bomb). In such an event, it is important that biological dosimetry laboratories are capable to respond adequately using cytogenetic assays in a triage mode, speeding up the analysis (i.e., with computer-assisted microscopy), and by networking with other laboratories. Therefore, there is an urgent need for updating existing knowledge, by producing documents/technical reports/manuals, by standardization of techniques,

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and by building of networks and initiating joint projects. Here, we report a linear-quadratic model of dose-response curve for three techniques such as DC assay in metaphases, FISH in metaphases, and micronuclei (MNs) assay in binucleated cells, and the Pearson's correlation analysis was performed to understand the correlation among the results from three biodosimetry techniques.

Materials and Methods

Dicentric chromosome assay

Human peripheral blood was obtained from three apparently healthy donors (32-, 33-, and 39-year-old males) under the Institutional Ethics Review Board procedures. Blood from each donor was placed in heparinized Vacutainer tubes (Becton Dickinson, USA). Whole blood was aliquoted into nine separate tubes (one as a control and the others for acute single exposure to doses of 0.1, 0.25, 0.5, 0.75, 1, 2, 4, and 6 Gy gamma rays (^{60}Co source-Atomic Energy of Canada, Ltd., Ontario, Canada) at a dose rate of 0.5 Gy/min. The procedures for lymphocyte culture were followed according to the description in the International Atomic Energy Agency technical report 405.[4] In brief, the exposed cells were cultured in 10 ml of Roswell Park Memorial Institute (RPMI) 1640 supplemented with 20% fetal bovine serum (FBS), 100 U/ml penicillin and 10 μg/ml streptomycin, and 30 μl/ml phytohemagglutinin (PHA) and kept in an incubator at 37°C in a 5% CO_2 humidified atmosphere for 48 h. To block the mitotic process of the cells at the metaphase stage, colcemid was added for the last 4 h of culture at a final concentration of 0.1 mg/ml. The harvested cells were treated with hypotonic 0.075 M KCl for 20 min and fixed with Carnoy's fixative (3:1 = methanol: acetic acid glacial) 3 times. Two slides for each sample were prepared, encoded, and then stained with 4% Giemsa and mounted. Mitotic cells on the slide were captured under Metafer System (MetaSystems, Germany). Scoring was done by a single scorer in complete metaphases. Tricentrics and tetracentrics were considered as two and three dicentric equivalents, respectively, and ring was skipped for dose-response curve.

Fluorescence *in situ* hybridization

Two slides were prepared for each sample, encoded, and then FISH assay was carried out under the manufacture's manual with mixture of chromosome probes #1, #4, and #8 with fluorescein isothiocyanate and count stained with 4',6-diamidino-2-phenylindole. The stained mitotic cells on the slide were captured under Metafer System. Scoring was done by a single scorer in complete metaphases.

Micronuclei assay in binucleated cells

Human peripheral blood was obtained from normal three healthy donors (composed of 41-, 44-, 50-year-old females) under the Institutional Ethics Review Board procedures. The heparinized whole blood was divided into seven containers - one group as a control and the others for single exposure to 0, 0.1, 0.25, 0.5, 0.75, 1, and 2 Gy gamma at a dose rate of 0.5 Gy/min. Exposed cells were cultured in 10 ml of RPMI 1640 supplemented with 20% FBS, 30 μl/ml PHA, 100 U/ml penicillin and 100 μg/ml streptomycin and kept in an incubator at 37°C in a 5% CO_2 humidified atmosphere for 43 h. Cytochalasin B was added for the last 29 h of culture at a final concentration of 3 ug/ml to block the cytoplasmic division. The harvested cells were treated with hypotonic 0.075 M KCl for 3 min and fixed with Carnoy's fixative (3:1 = methanol: acetic acid glacial) 3 times. Two slides were prepared for each sample, encoded, and then stained with 4% Giemsa and mounted. Binucleated cells on the slide were captured under Metafer System. A cell was considered as aberrant if it had over one micronucleus from each binucleated cell. Scoring was done by a single scorer.

Statistics

Statistical analysis of the data for linear-quadratic model of dose-response curve of three techniques was performed using Dose Estimate software version 4.1. To verify dose-response relationship, we performed Pearson's correlation analysis between sets of the results of biodosimetry assays. Pearson's correlation coefficient measures linear correlation between the pairwise DC, FISH, and MN. The correlation coefficient value 1 means strong positive linear relation, 0 means no correlation, and −1 means total negative correlation. *P* value for each correlation coefficient is given to decide its significance between sets of biodosimetry assays.

Results and Discussion

Dicentric chromosome assay

The results of the frequencies of chromosome aberrations in mitotic cells after irradiation by 0, 0.1, 0.25, 0.5, 0.75, 1, 2, 4, and 6 Gy gamma rays as a single dose are presented in Table 1 and Figure 1. As shown in Table 1, there is a significant difference between nonirradiated and irradiated group. The dose-response curve was fitted by Dose Estimate software version 4.1 as shown in Figure 1. The DC yield was fitted with a linear-quadratic model as the following equation: $Y = c + bD + aD^2$ where Y is the yield of dicentric frequency, D is absorbed dose in Gy, a is the corresponding quadratic coefficient, b is the linear coefficient, and c is the background frequency. Dose-response curve of DC fitted using a linear quadratic equation ML_Linear-Quadratic_Fit_Yield = 0.0049 (±0.0020) +0.0159 (±0.0073) × D + 0.0234 (±0.0033) × D^2 is showed in Figure 1 with the 95% lower confidence limit and upper confidence limit. Weighted Chi-squared is 516.7000, degrees of freedom is 6, and *P* value for goodness of fit is 0.0000. *P* values for coefficients (χ-test): *P*_A is 0.0463, *P*_alpha 0.0727, and *P*_beta

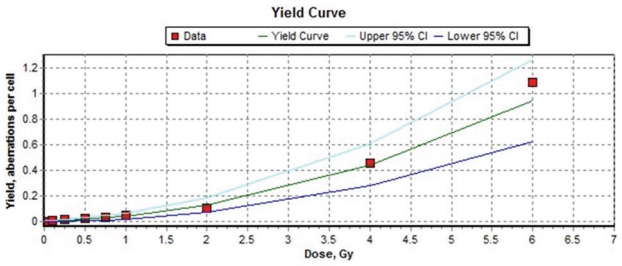

Figure 1: Dose-response curve for dicentric chromosomes in human peripheral blood lymphocytes following irradiation with ^{60}Co γ-rays to doses of 0.1 to 2 Gy at a dose rate of 0.5 Gy/min. The curve was generated by Dose Estimate software version 4.11

is 0.0004. Correlation coefficient, *r* is 0.9985. Based on the resulting coefficients, the dose estimation was done by input, the number of aberrations observed and cells scored.

Chromosome translocation (fluorescence *in situ* hybridization) assay

Frequencies of chromosome translocations in mitotic cells after irradiation with 0, 0.1, 0.25, 0.5, 0.75, 1, 2, 4, and 6 Gy gamma rays are shown in Table 2 and Figure 2. There was a statistically significant difference between the nonirradiated and irradiated group. Dose-response curve of chromosome translocations fitted using a linear quadratic equation ML_Linear-Quadratic_Fit_Yield = 0.0012 (±0.0004) + 0.0109 (±0.0028) × D + 0.0418 (±0.0013) × D^2 is showed in Figure 2 with the 95% lower confidence limit and upper confidence limit. Weighted Chi-squared is 118.8000, degrees of freedom is 6, and *P* value for goodness of fit is 0.0000. *P* values for coefficients (χ-test): *P*_A is 0.0375, *P*_alpha is 0.0084, and *P*_beta is 0.0000. Correlation coefficient, *r* is 0.9998. Based on the resulting coefficients, the dose estimation was done by computing the number of aberrations observed and cells scored.

Micronuclei assay in binucleated cells

The results of the frequencies of MNs in binucleated cell after irradiation by 0, 0.1, 0.25, 0.5, 0.75, 1, and 2 Gy gamma rays as a single dose are shown in Table 3 and Figure 3.

Table 1: Distribution and frequencies of dicentric chromosome aberrations in human peripheral blood lymphocytes following irradiation with ^{60}Co (γ-rays) at a dose rate of 0.5 Gy/min to doses of 0.1 to 6 Gy

Dose (Gy)	Scored cells	DC0	DC1	DC2	DC3	DC4	DC5	DC6	DC7	Total DC	DC/cell
0	2035	2028	7							7	0.0034
0.1	1960	1947	13							13	0.0066
0.25	1321	1304	15	2						19	0.0144
0.5	1608	1577	28	3						34	0.0211
0.75	1970	1905	61	4						69	0.0350
1	1852	1781	60	10		1				84	0.0454
2	1983	1805	144	33	1					213	0.1074
4	689	474	133	67	11	3	1			317	0.4601
6	355	169	60	75	33	15	2		1	386	1.0873

DC: Dicentric chromosome

Table 2: Distribution and frequencies of chromosome translocations (Ch #1, 4, and 8, fluorescent *in situ* hybridization) in human peripheral blood lymphocytes following irradiation with ^{60}Co (γ-rays) at a dose rate of 0.5 Gy/min to doses of 0.1-6 Gy

Dose (Gy)	Scored cells	Cells containing TRs	TR0	TR1	TR2	TR3	TR4	TR5	TR6	Total TRs	TR/cell
0.00	2379	2	2377	1	1					3	0.001
0.10	1883	5	1878	5						5	0.003
0.25	2257	14	2243	14						14	0.006
0.50	1796	25	1771	24	1					26	0.014
0.75	1922	59	1863	59						59	0.031
1.00	2338	129	2209	120	9					138	0.059
2.00	916	169	747	152	14	3				189	0.206
4.00	1124	499	625	314	114	50	20	1		777	0.691
6.00	648	502	146	205	141	101	40	11	4	1029	1.588

TRs: Translocations

Table 3: Distribution and frequencies of micronuclei in binucleated cell in human peripheral blood lymphocytes following irradiation with ^{60}Co (γ-rays) at a dose rate of 0.5 Gy/min to doses of 0.1-2 Gy

Dose (Gy)	Total counted binucleated cells	Total number of MNs	Binucleated cells with MNs								Frequency (total number of MNs/ total counted binucleated cells)
			1	2	3	4	5	6	7	Total	
0	3000	96	84	6						90	0.032
0.1	3000	152	130	11						141	0.051
0.25	3000	207	153	21	4					178	0.069
0.5	3000	292	223	23	6		1			253	0.097
0.75	3000	335	253	33	4	1				291	0.112
1	3000	558	389	61	12	1			1	464	0.186
2	3000	1209	779	166	24	2	1	1	1	974	0.403

MNs: Micronuclei

Table 4: Pearson correlation analysis between sets of biodosimetry techniques

	Pearson correlation coefficients ($P>\|r\|$ under H_0:p=0)		
	DC	TR_FISH	MN
DC	1.0000	0.9920<0.0001	0.99440<0.0001
TR	0.9920<0.0001	1.0000	0.99011<0.0001
MN	0.99440<0.0001	0.99011<0.0001	1.0000

MN: Micronuclei, TR: Translocation, DC: Dicentric chromosome, FISH: Fluorescent *in situ* hybridization

Data from Table 3 show significant difference among nonirradiated and irradiated group. Dose-response curve of MNs in binucleated cells was fitted using a linear quadratic equation ML_Linear-Quadratic_Fit_Yield = 0.0367 (±0.0056) + 0.0937 (±0.0226) × D + 0.0445 (±0.0136) × D^2 is showed in Figure 3 with the 95% lower confidence limit and upper confidence limit. Weighted Chi-squared is 16.4400, degrees of freedom is 4, and *P* value for goodness of fit is 0.5012. *P* values for coefficients (χ-test): *P*_A is 0.0027, *P*_alpha is 0.0144, *P*_beta is 0.0307. Correlation coefficient, *r* is 0.9968. Based on the resulting coefficients, the dose estimation was done by input, the number of aberrations observed and cells scored.

Comparison of the biodosimetry techniques following radiation exposure

Table 4 shows the significant Pearson's correlation coefficient *r* and *P* value. There appears to be a very strong positive correlation between the DC and translocation_FISH (TR_FISH) techniques ($r = 0.9920$, $P < 0.0001$) [Table 4 and Figure 4a]. The scatter plot suggests a relationship between the DC and TR_FISH biodosimetry techniques, with larger values of DC tending to be associated with larger values of TR_FISH. The DC and MN techniques have strong positive correlation ($r = 0.99440$, $P < 0.0001$) [Table 4 and Figure 4b]. The scatter plot suggests a relationship between the DC and MN biodosimetry techniques, with larger values of DC tending to be associated with larger values of MN. The TR_FISH and MN techniques have strong positive correlation ($r = 0.99011$, $P < 0.0001$) [Table 4 and Figure 4c]. The scatter plot suggests a relationship between the TR_FISH and MN biodosimetry techniques, with larger values of TR_FISH tending to be associated with larger values of MN.

Conclusion

Our data showed that the DC and FISH, the DC and MN, the FISH and MN biodosimetry techniques were linearly correlated very strongly [Table 4 and Figure 4].

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Conflicts of interest

There are no conflicts of interest.

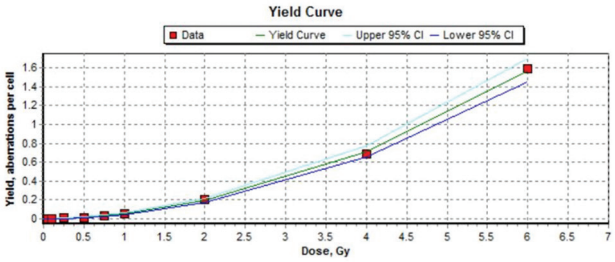

Figure 2: Dose-response curve for chromosome translocations (Ch #1, 4, and 8) in human peripheral blood lymphocytes following irradiation with ^{60}Co γ-rays to doses of 0.1 to 2 Gy at a dose rate of 0.5 Gy/min. The curve was generated by Dose Estimate software version 4.1

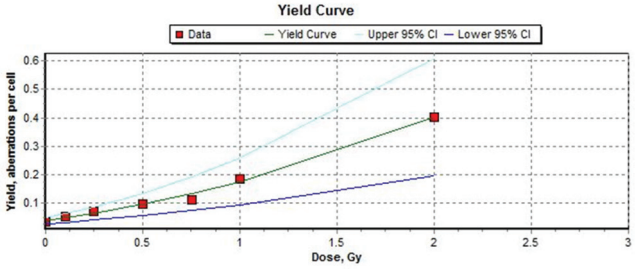

Figure 3: Dose-response curve for micronuclei in binucleated cell (micronuclei) in human peripheral blood lymphocytes following irradiation with ^{60}Co γ-rays to doses of 0.1 to 2 Gy at a dose rate of 0.5 Gy/min. The curve was generated by Dose Estimate software version 4.1

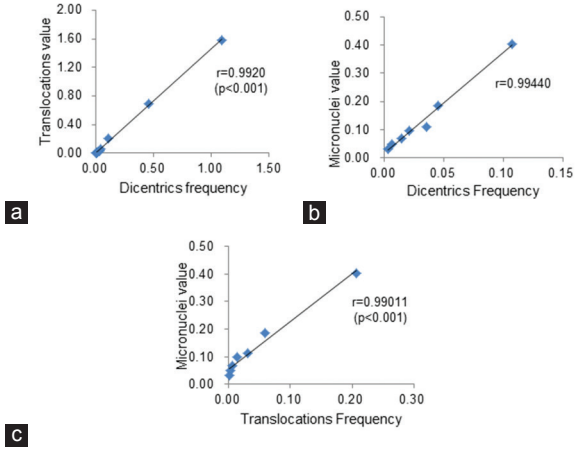

Figure 4: Scatter plot of the results from the DC and the FISH biodosimetry techniques (a), the DC and the MN biodosimetry technique (b), and the TR_FISH and the MN biodosimetry technique (c). DC: Dicentric chromosome, TR_FISH: Translocation_fluorescence *in situ* hybridization, MN: Micronuclei in binucleated cells

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