Journal of Radiation Research, 2013, 54, 863–871
doi: 10.1093/jrr/rrt032 Advance Access Publication 17 April 2013

Assessment of simulated high-dose partial-body irradiation by PCC-R assay

Ivonne ROMERO[1,*], Omar GARCÍA[1], Ana I. LAMADRID[1], Eric GREGOIRE[2], Jorge E. GONZÁLEZ[1], Wilfredo MORALES[3], Cécile MARTIN[2], Joan-Francesc BARQUINERO[2] and Philippe VOISIN[2]

[1]Centro de Protección e Higiene de las Radiaciones, Calle 20 No. 4113, e/41 y 47, Playa, CP 11300, La Habana, Cuba
[2]Institut de Radioprotection et de Sûreté Nucléaire, BP 17, 92262 Fontenay-aux-Roses, France
[3]Facultad de Matemática y Computación, Universidad de La Habana, San Lázaro y L, Plaza de la Revolución, CP 10400, La Habana, Cuba
*Corresponding author. Centro de Protección e Higiene de las Radiaciones, Calle 20 No. 4113, e/41 y 47, Playa, CP 11300, La Habana, Cuba. Tel: +53 7 682 9571; Fax: +53 7 682 9573; Email: ivonne@cphr.edu.cu

(Received 8 November 2012; revised 11 March 2013; accepted 12 March 2013)

The estimation of the dose and the irradiated fraction of the body is important information in the primary medical response in case of a radiological accident. The PCC-R assay has been developed for high-dose estimations, but little attention has been given to its applicability for partial-body irradiations. In the present work we estimated the doses and the percentage of the irradiated fraction in simulated partial-body radiation exposures at high doses using the PCC-R assay. Peripheral whole blood of three healthy donors was exposed to doses from 0–20 Gy, with ^{60}Co gamma radiation. To simulate partial body irradiations, irradiated and non-irradiated blood was mixed to obtain proportions of irradiated blood from 10–90%. Lymphocyte cultures were treated with Colcemid and Calyculin-A before harvest. Conventional and triage scores were performed for each dose, proportion of irradiated blood and donor. The Papworth's ***u*** test was used to evaluate the PCC-R distribution per cell. A dose-response relationship was fitted according to the maximum likelihood method using the frequencies of PCC-R obtained from 100% irradiated blood. The dose to the partially irradiated blood was estimated using the Contaminated Poisson method. A new D_0 value of 10.9 Gy was calculated and used to estimate the initial fraction of irradiated cells. The results presented here indicate that by PCC-R it is possible to distinguish between simulated partial- and whole-body irradiations by the ***u***-test, and to accurately estimate the dose from 10–20 Gy, and the initial fraction of irradiated cells in the interval from 10–90%.

Keywords: PCC-R; partial irradiation; D_0 value; high doses radiation; Calyculin A

INTRODUCTION

Information on the absorbed dose and its distribution in the body is of great importance for evaluating accidental radiation overexposure events. It influences decisions for immediate medical treatment, further health care and prognosis of exposed individuals. Accident reconstruction, skin reactions, and other indicators, including the dicentric assay, can provide information about the homogeneity of the exposure [1].

For dose-assessment in cases of partial-body exposure, the use of the dicentric assay is based on the observed dicentrics per cell distribution. After a homogenous exposure the distribution follows the Poisson distribution, while overdispersion is typical after partial-body irradiation. The fraction of the irradiated body and the dose received can be estimated using appropriated equations [1–3]. This approach, developed for use with the dicentric assay, needs to be evaluated as a tool for dose-assessment in cases of partial-body exposures using the newly emerging cytogenetic indicators.

The method of premature chromosome condensation (PCC), by fusion [4] or using chemical inhibitors of phosphatases 1 and 2A [5, 6], offers several advantages in cytogenetic dosimetry. Probably the most recently highlighted is the potential to overcome the problem of poor cell

proliferation after high doses of radiation. By a chemically induced PCC technique several endpoints can be used, excess fragments [6], aberrations detected by chromosome painting [7], or in the simplest version, scoring PCC rings (PCC-R) [8]. Dose-response curves up to 20 Gy using low-LET radiation [8–11] and up to 4 Gy using high-LET radiation [9] have been obtained using the PCC-R assay. This technique was successfully applied for estimating the high doses at the Tokaimura accident [12].

The possibility of applying the PCC-R assay to evaluate simulated partial-body exposures has been tested recently [11]. In that study the PCC ring and dicentric assays were used in parallel in a triage mode, scoring 30 dicentrics or 50 metaphases for dicentrics, and 50 rings or 300 PCC cells in the PCC-R assay. Whole- and partial-body irradiations up to 13 Gy were simulated. Under these circumstances neither assay was successful in identifying partial-body irradiation. The most probable reason for this was the low numbers of cells scored in the triage mode. A limitation of the triage mode in the evaluation of partial-body irradiation was highlighted recently when the dicentric assay failed in the identification of overdispersion and dose-estimation during the assessment of a patient partially exposed to radiation [13].

The aim of the present study was to test the potential to use the PCC-R assay in the assessment of partial-body exposures using the approaches already existing for the dicentric assay. These include distinguishing between total and partial irradiation, estimating partial-body dose and estimating the initial fraction of irradiated blood. Considering the applicability of the PCC-R assay for high doses, we simulated partial-body exposures between 10 and 90% and focused our attention on the dose-interval of 10–20 Gy. A proposed D_0 value has been calculated in order to determine the initial fraction of irradiated cells to be used with the PCC-R assay.

MATERIALS AND METHODS

Blood irradiation and simulated partial-body irradiation

Peripheral blood samples from three healthy donors were obtained with informed consent and according to the institutional ethics procedures. Samples were exposed at doses of 0, 1, 5, 7.5, 10, 15 and 20 Gy (dose-rate 0.5 Gy/min) using a source of ^{60}Co gamma radiation. IAEA recommendations were followed during irradiations [1]. To simulate partial-body exposures, irradiated blood at 10, 15 and 20 Gy was mixed with non-irradiated blood from the same donor to final proportions of 10, 25, 50, 75, 90 and 100%. Additionally the blood irradiated at 1, 5, and 7.5 Gy was mixed with non- irradiated blood to simulate 50% partial-body irradiation. All the data, simulating partial and whole body irradiations were used for D_0 estimation.

Lymphocyte culture, PCC-R assay and scoring criteria

The PCC-R assay was conducted as described by Lamadrid *et al.* [9]. This protocol was selected because it was demonstrated that rings with Poisson distribution are obtained after total-blood irradiation. In all cases, 0.5 ml of peripheral whole blood was cultured for 48 h in 5 ml of RPMI 1640 medium containing L-glutamine, 20% foetal calf serum and 1% phytohaemaglutinin (PHA). Colcemid (0.05 μg/ml) was added 24 h after the beginning of the culture, and Calyculin A (50 nM) was added 1h before the harvest. Cultured cells were treated with a hypotonic solution of KCl (0.075M) for 8 min at 37°C and fixed in three changes of fixative (methanol:acetic acid, 3:1 v/v). Finally 30 μl of the final cell suspension was dropped onto the slides, air-dried and stained with 4% Giemsa solution.

The presence of rings in PCC cells in G1, G2, metaphase and anaphase was scored as described by Lamadrid *et al.* [9]. Rings were counted when they displayed an open circle in the middle, or when they were perfectly round and their size exceeded the width of the chromatids in that PCC cell. In G2 or anaphase PCC cells, when two identical rings were observed only one was recorded as it was considered that the rings were originally from the same chromosome. See Supplementary Figure. As a rule the conventional or full score (analysis of 500 PCC cells or up to 100 rings) was made for each datapoint. In order to simulate a triage scoring, the first 300 PCC cells or 50 rings scored were considered.

Statistical analysis

Dose-response curve

A dose-response curve was fitted by the maximum likelihood method in the simulated whole-body dose interval from 0–20 Gy using the separate data of the three donors. The difference between donors was tested using the F test for comparing curves and the Student t- test for comparing frequencies.

PCC ring per cell distribution

The distributions of PCC rings per cell were evaluated using the DoseEstimate software [14]. The normalized unit u of the dispersion index (σ^2/Y) was analyzed for each dose and each proportion of irradiated blood, assuming Poisson distribution if $u \leq 1.96$, and overdispersion if $u > 1.96$.

The percentage of correct SPBI identifications ($\%SPBI_{ID}$) was calculated as follows:

$$\%SPBI_{ID} = \frac{\#SPBI_{u>1.96}}{\#SPBI_T} \bullet 100,$$

where $\#SPBI_{u>1.96}$ is the number of rings' distribution by cell that do not follow the Poisson distribution, and $\#SPBI_T$ is the total number of rings' distributed by the analyzed cell.

Table 1. The frequencies and intercellular distributions of PCC rings measured in the three donors for all doses and proportions of irradiated blood tested, in 500 PCC cells or up to 100 rings when possible.

irradiated blood	Dose (Gy)	Donor	Cells	Rings	Distribution of rings						Y ± SE	σ^2/Y	u
					0	1	2	3	4	5			
0%	0	1	301	1	300	1	0	0	0	0	0.003 ± 0.007	1.00	***0.00***
		2	360	0	360	0	0	0	0	0	0.000 ± 0.000	0.00	***0.00***
		3	658	1	657	1	0	0	0	0	0.002 ± 0.009	0.00	***0.00***
10%	10	2	500	22	485	10	4	0	1	0	0.822 ± 0.014	1.87	14.05
		3	500	11	491	7	2	0	0	0	0.416 ± 0.006	1.34	5.70
	15	2	500	15	491	6	0	3	0	0	1.126 ± 0.016	2.17	19.20
		3	500	18	487	9	3	1	0	0	0.690 ± 0.011	1.63	10.30
	20	2	500	14	492	4	2	2	0	0	1.247 ± 0.016	2.12	18.34
		3	500	6	496	2	2	0	0	0	0.874 ± 0.008	1.66	11.39
25%	10	1	500	12	490	8	2	0	0	0	0.376 ± 0.006	1.31	5.15
		2	500	30	478	17	3	1	1	0	0.656 ± 0.014	1.74	11.94
		3	500	20	483	14	3	0	0	0	0.334 ± 0.007	1.26	4.25
	15	1	500	6	494	6	0	0	0	0	0.000 ± 0.000	0.99	***–0.17***
		2	500	32	478	13	8	1	0	0	0.803 ± 0.017	1.63	10.06
		3	500	19	488	8	3	0	0	1	1.002 ± 0.016	2.34	21.67
	20	1	500	3	497	3	0	0	0	0	0.000 ± 0.000	1.00	***–0.08***
		2	500	51	468	18	11	1	2	0	1.018 ± 0.026	1.92	14.70
		3	500	18	489	6	4	0	1	0	1.081 ± 0.016	2.08	17.54
50%	1	1	500	2	498	2	0	0	0	0	0.000 ± 0.000	1.00	***–0.04***
		2	500	3	497	3	0	0	0	0	0.000 ± 0.000	1.00	***–0.08***
		3	500	6	494	6	0	0	0	0	0.000 ± 0.000	0.99	***–0.17***
	5	1	447	16	431	16	0	0	0	0	0.000 ± 0.000	0.97	***–0.52***
		2	500	55	448	49	3	0	0	0	0.113 ± 0.006	1.00	***0.02***
		3	500	32	474	23	1	1	1	0	0.431 ± 0.010	1.56	9.05
	7.5	1	522	36	490	28	4	0	0	0	0.240 ± 0.007	1.16	2.54
		2	500	84	433	53	11	3	0	0	0.471 ± 0.018	1.31	4.94
		3	500	36	469	26	5	0	0	0	0.307 ± 0.009	1.21	3.34
	10	1	494	29	468	24	1	1	0	0	0.223 ± 0.006	1.22	3.51
		2	500	73	440	49	9	2	0	0	0.406 ± 0.015	1.27	4.25
		3	500	83	439	42	16	3	0	0	0.651 ± 0.023	1.44	6.98
	15	1	500	41	471	18	10	1	0	0	0.738 ± 0.018	1.56	8.88
		2	500	119	418	53	22	6	1	0	0.798 ± 0.033	1.54	8.54
			500	56	467	18	10	2	3	0	1.171 ± 0.031	2.11	17.64
	20	1	470	43	441	16	12	1	0	0	0.847 ± 0.022	1.61	9.45
		2	500	112	430	44	15	7	3	1	1.027 ± 0.039	1.92	14.64
		3	500	30	479	14	6	0	1	0	0.761 ± 0.016	1.74	11.94
75%	10	1	454	62	399	49	5	1	0	0	0.245 ± 0.011	1.12	***1.88***
		2	400	112	319	57	19	3	2	0	0.687 ± 0.035	1.44	6.21
		3	350	111	258	74	17	1	0	0	0.388 ± 0.026	1.05	***0.61***
	15	1	500	114	419	53	23	5	0	0	0.727 ± 0.030	1.44	7.01
		2	300	163	194	61	35	8	2	0	0.933 ± 0.073	1.33	4.08
		3	465	150	365	64	24	10	2	0	0.874 ± 0.043	1.56	8.57
	20	1	351	80	297	34	16	2	2	0	0.845 ± 0.040	1.63	8.34
		2	300	109	225	45	26	4	0	0	0.801 ± 0.052	1.34	4.16
		3	500	96	437	38	20	3	1	1	0.911 ± 0.033	1.75	11.89
90%	10	1	336	84	273	47	12	3	1	0	0.606 ± 0.033	1.40	5.17
		2	300	124	204	71	22	3	0	0	0.536 ± 0.040	1.09	***1.11***
		3	131	45	100	21	6	4	0	0	0.798 ± 0.077	1.47	3.81

Continued

Table 1. *Continued*

irradiated blood	Dose (Gy)	Donor	Cells	Rings	Distribution of rings						Y ± SE	σ^2/Y	u
					0	1	2	3	4	5			
	15	1	175	60	126	39	9	1	0	0	0.420 ± 0.40	1.06	***0.59***
		2	200	113	124	46	24	5	1	0	0.854 ± 0.084	1.24	2.38
		3	200	113	123	51	19	5	1	1	0.823 ± 0.082	1.33	3.27
	20	1	165	78	109	39	13	3	1	0	0.704 ± 0.072	1.25	2.30
		2	250	110	170	55	20	5	0	0	0.675 ± 0.055	1.20	2.25
		3	221	104	154	41	17	7	2	0	0.955 ± 0.080	1.50	5.25
100%	1	1	510	7	503	7	0	0	0	0	0.014 ± 0.005	0.99	***–0.20***
		2	500	8	492	8	0	0	0	0	0.016 ± 0.011	0.99	***–0.24***
		3	500	8	492	8	0	0	0	0	0.016 ± 0.009	0.99	***–0.24***
	5	1	248	35	215	31	2	0	0	0	0.141 ± 0.003	0.98	***–0.26***
		2	500	107	404	86	9	1	0	0	0.214 ± 0.014	1.01	***0.20***
		3	500	87	426	62	11	1	0	0	0.174 ± 0.008	1.15	2.39
	7.5	1	309	100	226	68	13	2	0	0	0.324 ± 0.003	1.06	***0.75***
		2	300	114	208	71	20	1	0	0	0.380 ± 0.003	1.03	***0.33***
		3	300	114	210	71	14	5	0	0	0.380 ± 0.005	1.13	***1.63***
	10	1	267	107	183	66	13	5	0	0	0.401 ± 0.009	1.13	***1.47***
		2	200	137	103	62	31	3	1	0	0.685 ± 0.015	0.99	***–0.09***
		3	200	110	114	66	16	4	0	0	0.550 ± 0.011	0.96	***–0.36***
	15	1	163	100	88	53	19	3	0	0	0.613 ± 0.011	0.95	***–0.43***
		2	102	102	42	28	24	6	2	0	1.000 ± 0.018	1.07	***0.49***
		3	150	108	75	48	22	4	1	0	0.720 ± 0.012	1.03	***0.24***
	20	1	153	101	79	53	16	4	1	0	0.660 ± 0.011	1.02	***0.17***
		2	150	110	71	55	20	1	3	0	0.733 ± 0.017	1.02	***0.16***
		3	132	107	66	36	21	8	0	1	0.811 ± 0.018	1.23	***1.84***

The *u* value was used to assess the partial irradiation.

D_0 value calculation

The surviving fraction (*S*) was calculated using the equations reported by Lloyd and coworkers [15] and Matsubara and coworkers [16]. The frequencies of rings per cell necessary for the calculation of *S* were obtained from simulated whole- and partial-body irradiation. Data from all the proportions of the simulated partial-body irradiation were used following Matsubara's method, while data simulating 50% partial-body irradiation was used when Lloyd's method was applied. The *in vivo* volume of the body originally used by Matsubara in the equation for the estimation of the survival fraction [16] was replaced in our simulated partial body irradiation (SPBI) by the *in vitro* volume used. The D_0 was estimated using the linear regression between Ln(*S*) and the dose. This linear regression was constrained to go through the 100% survival point at zero dose. The detailed procedure is presented in the Supplementary Material.

Estimation of the exposed fraction and its dose

The exposed fraction and its dose were estimated by applying the Contaminated Poisson method originally proposed for the dicentric assay [1–2] using the dose-response calibration curve established in this study, and the yield of rings estimated in the irradiated fraction for each donor. The detailed procedure can be found in the Supplementary Material. Estimated doses within 30% of the true dose were considered as acceptable [17], and estimated irradiated fractions within 10% of the true irradiated fraction were also considered as acceptable.

The percentage of correct fraction estimations ($\%F_{correct}$) was calculated as follows:

$$\%F_{correct} = \frac{\#F_{10\%}}{\#F_T} \bullet 100,$$

where $\#F_{10\%}$ is the number of irradiated fractions estimated within 10% of the true irradiated fraction, and $\#F_T$ is the total number of irradiated fractions estimated.

The percentage of correct dose estimations ($\%D_{correct-SPBI}$) was calculated as follows:

$$\%D_{correct-SPBI} = \frac{\#D_{SPBI-30\%}}{\#D_{SPBI-T}} \bullet 100,$$

where $\%D_{SPBI-30\%}$ is the number of estimated SPBI doses within 30% of the true dose, and $\%D_{SPBI-T}$ is the total number of estimated SPBI doses.

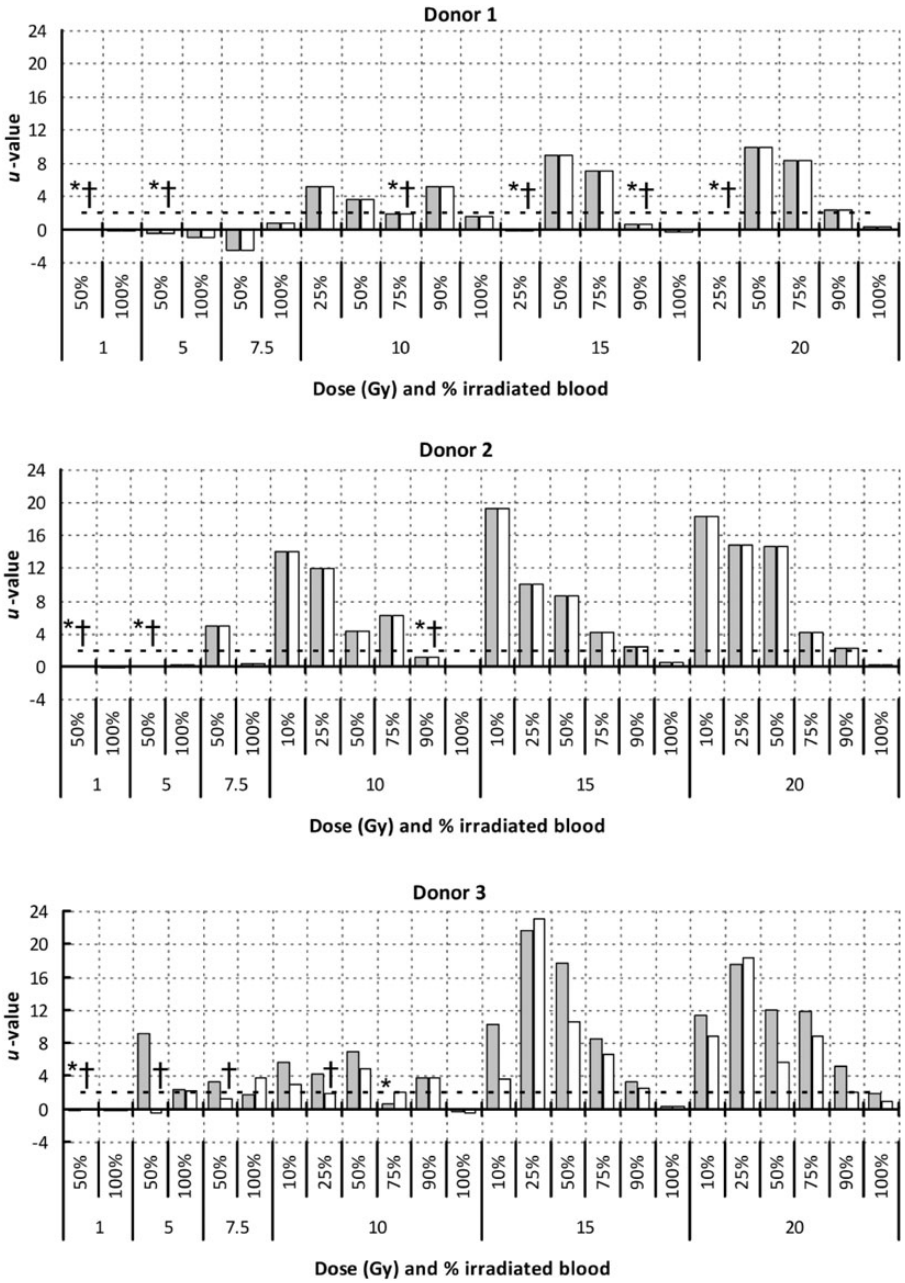

Figure 1. Posison distribution evaluated in the three donors by *u*-value for conventional (grey bars) (500 PCC cells or up to 100 rings) or triage (white bars) (300 PCC cells or up to 50 rings) scored. All proportions and doses of irradiated blood are presented. Misclassified SPBI have been highlighted: in 500 PCC cells (asterisks) and in the first 300 PCC cells (daggers).

RESULTS

Identification of simulated partial-body irradiation

Table 1 shows the number of PCC cells and rings scored, the distribution of rings among scored cells, the frequency of rings, the dispersion index (σ^2/Y) and the *u* value, for the three donors and all tested doses and proportions of irradiated blood. As can be seen in the simulated whole-body irradiation, only one of 18 rings per cell distribution does not conform to the Poisson distribution, while in SPBI, in 40 of the 51 cases the *u* values indicate overdispersion.

Analyzing each donor individually (Table 1) we obtain a correct identification of the SPBI (i.e. $u > 1.96$) in 60% of samples from donor 1 (no data were obtained for the 10% fraction), in 83% of samples from donor 2, and in 88% of the samples from donor 3. Five of the 11 misidentifications of SPBI correspond to the two lowest doses used, 1 and 5 Gy. Figure 1 shows the *u* values for each donor at each

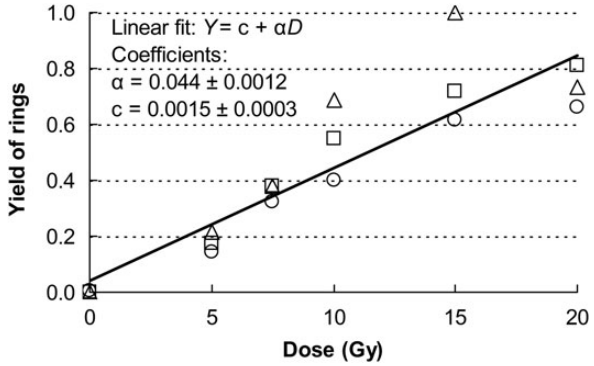

Figure 2. Linear relationship between the PCC rings frequency and dose. The regression was made taking in account the individual data of each donor (Donor 1 (open circles); Donor 2 (open triangles); Donor 3 (open squares).

dose and proportion of irradiated blood. Generally, the *u* value was higher in the lower fraction of SPBI with a tendency to decrease with the increase in the percentage of irradiated blood. The number of correct identifications of SPBI decreased to 68% when the triage mode was simulated (i.e. scoring only 300 cells).

Dose estimation

Figure 2 shows the linear dose-response relationship between the frequency of rings and the dose. As can be seen, there is apparent saturation in the PCC-ring frequency of donor 2 after 15 Gy. Even so, the dose responses were statistically the same in the three donors according to the *F* test ($P > 0.05$). The estimated doses after SPBI are presented in Fig. 3. At 10 Gy 43% of estimated doses were classified as acceptable, with the 95% confidence intervals for all the doses estimated between 0 and 31 Gy. At 15 Gy 50% of the estimated doses were deemed acceptable with 95% confidence intervals for all the doses estimated between 1 and 44 Gy, while at 20 Gy 86% of the estimated doses were classified as acceptable within 95% confidence intervals for all the doses estimated between 0 and 48 Gy. Overall, 64% of all the estimated doses in SPBI were classified as acceptable. A tendency towards improved estimations with increase of dose and percentage of irradiated blood was observed.

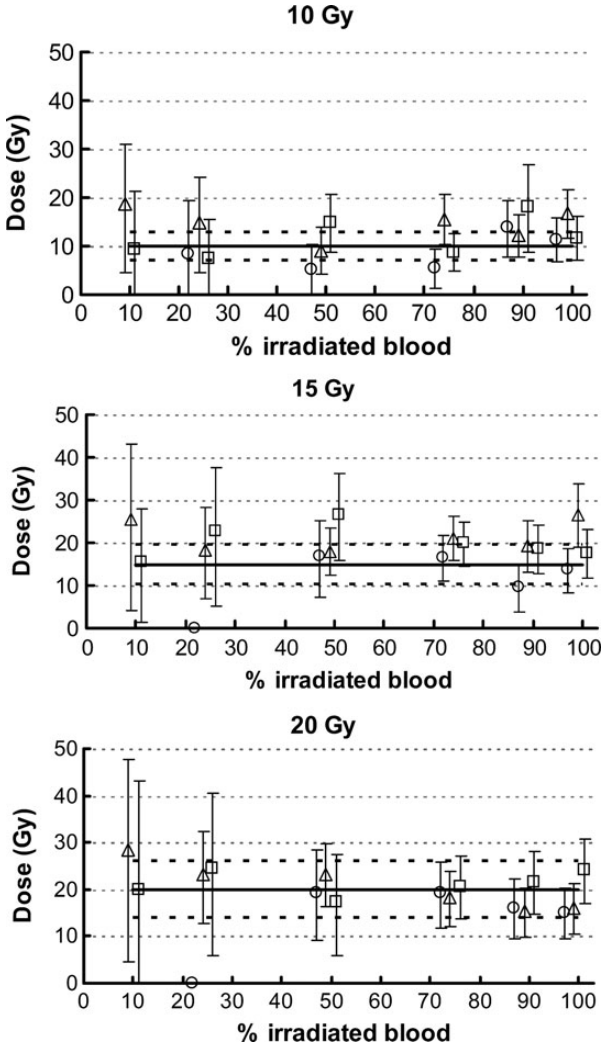

Figure 3. Estimates of dose ± 95% confidence limits for 10, 15 and 20 Gy blood irradiation, for the three donors tested (Donor 1 (open circles); Donor 2 (open triangles); Donor 3 (open squares). The solid line represents the real dose and the discontinuous line ±30% of the real dose. A decrease in the 95% confidence limits and an increase in the precision of the dose estimation have been obtained with increased proportion of irradiated blood.

Estimation of the irradiated fraction

D_0 value

Figure 4 shows the linear regression between Ln (*S*) and dose, the results previously reported for the dicentric assay [15] are also included for comparison. The D_0 values obtained were 10.2 Gy according to Lloyd *et al.* [15] and 10.9 Gy, according to Matsubara *et al.* [16]. Although no statistical difference (Student's t-test, $P > 0.05$) was found between the estimations of the irradiated fraction using the two D_0 values, more estimated fractions fell inside the acceptable range using the D_0 value of 10.9 Gy.

Irradiated fraction

The results obtained in the estimation of the irradiated initial fraction of blood for each donor are presented in Fig. 5. Approximately 60% of the estimated irradiated fractions were deemed as acceptable, and 62% of the 95% confidence intervals encompassed the true value. The lowest number of acceptable estimations (3 out of 9) was obtained at 50% SPBI, while 3 out of 6 estimations made at 10% SPBI, 5 out of 9 estimations at 25% SPBI, 6 out of 9

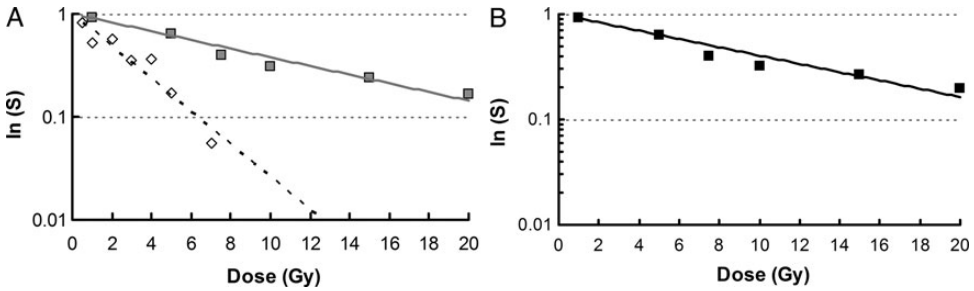

Figure 4. The logarithm of the cells with prematurely condensed chromosomes (PCC) cells from the SPBI cultures as a fraction of those PCC cells from cultures of blood 100% irradiated (S) plotted against the radiation dose. (**A**) Lloyd's data (open diamonds) (Lloyd *et al.*, 1973), present work's data following Lloyd's method (grey-filled squares), (**B**) present work's data following Matsubara's method (black-filled squares). The differences between the linear regression obtained with the dicentric and the PCC-R assay can be observed in graph A.

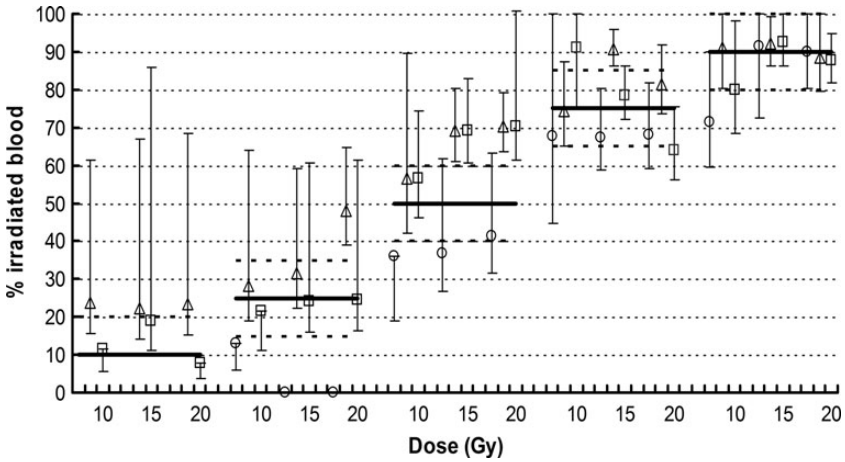

Figure 5. Estimates of the % of irradiated fraction ±95% confidence limits for 10, 25, 50, 75 and 90% SPBI, for the doses 10, 15 and 20 Gy, calculated by $D_{0-M} = 10.9$ Gy (Matsubara's method). The estimations made with $D_{0-L} = 10.2$ Gy (Lloyd's method) are not shown. The values of the three independent donors are: donor 1 (open circles); donor 2 (open triangles); donor 3 (open squares). The solid line represents the real irradiated fraction and the discontinuous line the ±10% interval of the real irradiated fraction.

estimations at 75% SPBI, and 8 out of 9 estimations at 90% SPBI were classified as acceptable. Considering donors individually, better estimations were obtained with donor 3 (~ 60% of correct estimations), while the worst results were obtained with donor 1 (~50 % correct), and a tendency to overestimation of the irradiated fraction.

DISCUSSION

Identification of simulated partial-body irradiation

The possibility to distinguish between partial- and whole-body irradiation by testing the distribution of PCC-rings among cells is critical for applying this assay in accident situations. The results obtained in the present study, scoring large numbers of cells or even limiting the analysis to simulate a triage, are similar to those reported using the dicentric assay, where from 86–100% of samples simulating whole-body irradiation and 60–100% of samples simulating partial-body irradiation were correctly identified by the *u* value [15, 17–19]. In the present work, approximately half of the SPBI misidentifications fell between 1 and 5 Gy, where the dose and the initial irradiated fraction assessment carry large statistical uncertainties due to the small number of aberrations found.

There are limited and contradictory earlier results on this issue for the PCC-R assay. It has been reported that the Poisson distribution applies for whole-irradiated blood, even after high-LET radiation exposure, using the PCC protocol employed in this study, i.e. with the inclusion of Colcemid [9–10], while non-Poisson distributions are more frequently reported using the PCC protocol without Colcemid treatment [11, 20]. By flow cytometry it has been reported that Colcemid treatment in PCC cultures accumulates metaphases, which increase the proportion of G2/M

PCC cells [8]. So with this protocol the analysis of PCC rings is probably closer to the classical dicentric assay where a Poisson distribution of rings is expected after whole-body irradiation [21].

Estimation of dose and irradiated fraction

In the present study we have analyzed blood from three donors and considered our previous unpublished data showing some differences between different donors' response to the same dose, and also looked at a report of small inter-individual variances [8]. The donors' responses were similar over the dose range used in this experiment. Nevertheless, the apparent saturation of the assay in one donor above 15 Gy, not obtained before using our protocol [9], supports previous findings of others authors, suggesting the possibility of assay saturation after 15 Gy [11] or 20 Gy [8], and this should be confirmed by extending the dose interval already tested with the PCC-R assay. This effect should be considered when doses >15 Gy are suspected, and it highlights the necessity of using appropriate numbers of donors for constructing calibration curves, or when simulating *in vitro* partial-body irradiation, despite the differences in scoring criteria [11] or PCC protocol used [22]. These and others factors, such as the dose rate, number of cells scored, etc. may determine the differences in coefficients for the linear relationship usually obtained for the PCC-R assay after high doses [8, 9, 11, 20].

The precision in dose and irradiated fraction estimations obtained in our simulation are close to those obtained in previous exercises using the dicentric assay [17, 19, 23]. In the evaluation of the outcome obtained using the PCC-R assay, it is advisable to use at least three donors separately for fitting the dose-response curve, while in previous studies with the dicentric assay usually only one donor was used, which led to larger uncertainties in the dose and irradiated fraction estimations.

D_0 value

Here we present the first attempt to calculate a D_0 value for the PCC-R assay, using the formulae proposed by Lloyd and Matsubara, for the dicentric assay.

This value should be considered when using the assay in accident situations where partial-body irradiation is suspected. The value obtained here is much higher than previous D_0 values reported for the dicentric assay for X-rays ($D_0 = 2.7$ [15], $D_0 = 3.8$ [19]) or gamma radiation ($D_0 = 3.5$ [16], $D_0 = 3.0$ [24]). This difference can be explained by the nature of the different endpoints measured in each assay. Whereas by the conventional dicentric assay D_0 is based on the ability of G_0-irradiated lymphocytes to reach metaphase, the D_0 measured here includes cells able to reach the G_2-phase of the cell cycle. Briefly, the formulae used are based on the initial proportion of irradiated/non-irradiated lymphocytes, and the frequencies of aberrations (dicentrics) per cell in cultures of partial- and total-irradiated blood. It is assumed that aberrations (dicentric or PCC-R) are formed in irradiated cells and the final yield is observed in a mixture of irradiated and unirradiated cells. The frequencies of both aberrations (dicentric and PCC-R) are dose-dependent and this is the most important variable in the calculation method.

Apparently there are no differences in the cell-cycling kinetics of lymphocytes having dicentrics or rings in either assay. Rodriguez *et al.* [25] demonstrated, using the PCC-R assay, that at the G_2/M checkpoint, there is minimal selection against complete chromosome elements (chromosome elements with both telomeric ends), which includes dicentrics and rings, and against dicentrics in general. So the only difference between the dicentric and the PCC-R assays is the increase in the frequency of rings per cell due to the fact that in the PCC-R assay, cells are analyzed in almost all phases of their cycle (G_1, metaphase, G_2), whereas in the dicentric assay only the cells able to reach metaphase are scored.

The few reports on D_0 value estimation for the dicentric assay [15, 16, 19] illustrate the limited attention that deriving this value has received, despite it being critical for the estimation of the irradiated fraction. The need to extend the experimental basis for D_0 value estimation was highlighted during an extensive intercomparison exercise using the dicentric assay, where the tendency to overestimation of the irradiated fraction was associated with the possible use of a low value of D_0 [17]. Difficulties in fraction estimation were also reported using another D_0 value derived for the dicentric assay [19]. It is expected that the use of different cell culture conditions, as well as differences in lymphocyte responses to PHA between donors, may influence the mitotic or PCC indices, and consequently, under similar irradiation conditions, the survival of the lymphocytes (which is measured by the number of metaphases or PCC cells) can vary. It seems reasonable to suggest that D_0 values should be derived individually by the different laboratories working in biological dosimetry, as recommended in the seminal paper on this topic [15].

CONCLUSION

The potential to distinguish partial from total irradiation by analyzing the distribution of PCC rings among cells was confirmed under the conditions used in the present study. In such circumstances it is possible to apply the pre-existing calculation tools developed for the dicentric assay for dose and fraction estimation and obtain results similar to those previously obtained for the dicentric assay. A D_0 value of 10.9 Gy gave the best results in fraction estimation. More experimental data from different laboratories, using different donors, radiation qualities and PCC protocols, should provide additional information on the

applicability of the PCC ring assay for the evaluation of partial-body irradiation.

FUNDING

This work was supported by a Type II grant from the International Atomic Energy Agency (CUB/10010) awarded to I.R.

ACKNOWLEDGEMENTS

The editorial assistance of D. Lloyd is greatly appreciated.

REFERENCES

1. IAEA. (ed) *Cytogenetic Dosimetry: Applications in Preparedness for and Response to Radiation Emergencies. EPR-Biodosimetry*. Vienna: International Atomic Energy Agency, 2011.
2. Dolphin G-W. Biological dosimetry with particular reference to chromosome aberration analysis. A review of methods. In: IAEA (ed). Handling of Radiation Accidents. *Proceedings of the International Symposium*, Vienna, 1969, 215–24.
3. Sasaki M-S, Miyata H. Biological dosimetry in atom bomb survivors. *Nature* (London) 1968;**220**:1189–93.
4. Pantelias G-E, Maillie H-D. A simple method for premature chromosome condensation induction in primary human and rodent cells using polyethylene glycol. *Somat Cell Genet* 1983;**9**:533–47.
5. Gotoh E, Tanno Y, Takakura K. Simple biodosimetry method for use in cases of high-dose radiation exposure that scores the chromosome number of Giemsa-stained drug-induced prematurely condensed chromosomes (PCC). *Int J Radiat Biol* 1995;**81**:33–40.
6. Gotoh E, Asakawa Y. Detection and evaluation of chromosomal aberrations induced by high doses of γ-irradiation using immunogold-silver painting of prematurely condensed chromosomes. *Int J Radiat Biol* 1996;**70**:517–20.
7. Durante M, Furusawa Y, Gotoh E. Technical report. A simple method for simultaneous interphase-metaphase chromosome analysis in biodosimetry. *Int J Radiat Biol* 1998;**74**:457–62.
8. Kanda R, Hayata I, Lloyd D-C. Easy biodosimetry for high-dose radiation exposures using drug-induced, prematurely condensed chromosomes. *Int J Radiat Biol* 1999;**75**:441–6.
9. Lamadrid A-I, García O, Delbos M *et al*. PCC-ring induction in human lymphocytes exposed to gamma and neutron irradiation. *J Radiat Res* 2007;**48**:1–6.
10. Lamadrid A-I, González J-E, García O *et al*. Prematurely condensed chromosome rings after neutron irradiation of human lymphocytes. *J Radiat Res* 2011;**52**:531–5.
11. Lindholm C, Stricklin D, Jaworska A *et al*. Premature chromosome condensation (PCC) assay for dose assessment in mass casualty accidents. *Radiat Res* 2010;**173**:71–8.
12. Hayata I, Kanda R, Minamihisamatsu M *et al*. Cytogenetical dose estimation for 3 severely exposed patients in the JCO criticality accident in Tokai-mura. *J Radiat Res* 2001;**42**: 149–55.
13. Ainsbury E-A, Livingston G-K, Abbott M-G *et al*. Interlaboratory variation in scoring dicentric chromosomes in a case of partial-body X-ray exposure: implications for biodosimetry networking and cytogenetic "triage mode" scoring. *Radiat Res* 2009;**172**:746–52.
14. Ainsbury E-A, Lloyd D-C. Dose estimation software for radiation biodosimetry. *Health Phys* 2010;**98**:290–5.
15. Lloyd D-C, Purrott R-J, Dolphin G-W. Chromosome aberration dosimetry using human lymphocytes in simulated partial body irradiation. *Phys Med Biol* 1973;**18**:421–31.
16. Matsubara S, Sasaki M-S, Adachit T. Dose–response relationship of lymphocytes chromosome aberrations in locally irradiated persons. *J Radiat Res* 1974;**15**:189–96.
17. Lloyd D-C, Edwards A-A, Prosser J-S *et al*. A collaborative exercise on cytogenetic dosimetry for simulated whole and partial body accidental irradiation. *Mutat Res* 1987;**179**:197–208.
18. Duran A, Barquinero J-F, Caballín M-R *et al*. Suitability of FISH painting techniques for the detection of partial-body irradiations for biological dosimetry. *Radiat Res* 2002;**157**:461–8.
19. Barquinero J-F, Barrios L, Caballín M-R *et al*. Biological dosimetry in stimulated *in vitro* partial irradiations. *Int J Radiat Biol* 1997;**71**:435–40.
20. Balakrishnan S, Shirsath K, Bhat N *et al*. Biodosimetry for high dose accidental exposures by drug induced premature chromosome condensation (PCC) assay. *Mutat Res* 2010;**699**:11–6.
21. Edwards A-A, Lloyd D-C, Purrot R-J. Radiation induced chromosome aberrations and the Poisson distribution. *Radiat Environ Biophys* 1979;**16**:89–100.
22. Stricklin D, Lindholm C, Jaworska A. Development and application of the PCC biodosimetry method in emergency preparedness. Final report of the NKS-B project Biodosimetry Applications in Emergency Preparedness-BioDos. 2006–2007 NKS-173 ISBN 978-87-7893-239-6 July 2008.
23. García O, Ramalho A-T, Di Giorgio M *et al*. Intercomparison in cytogenetic dosimetry among five laboratories from Latin America. *Mutat Res* 1995;**327**:33–9.
24. Lloyd D-C, Edwards A-A, Moquet J-E *et al*. The role of cytogenetics in early triage of radiation casualties. *Applied Radiation and Isotopes* 2000;**52**:1107–12.
25. Rodríguez P, Barquinero J-F, Duran A *et al*. (2009) Cells bearing chromosome aberrations lacking one telomere are selectively blocked at the G2/M checkpoint. *Mutat Res 2009*;**670**:53–8.