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Rapid assessment of high-dose radiation exposures through scoring of cell-fusion-induced premature chromosome condensation and ring chromosomes



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abstract

Analysis of premature chromosome condensation (PCC) mediated by fusion of G0-lymphocytes with mitotic CHO cells in combination with rapid visualization and quantification of rings (PCC-Rf) is proposed as an alternative technique for dose assessment of radiation-exposed individuals. Isolated lymphocytes or whole blood from six individuals were irradiated with 5, 10, 15 and 20 Gy at a dose rate of 0.5 Gy/min. Following either 8- or 24-h post-exposure incubation of irradiated samples at 37 °C, chromosome spreads were prepared by standard PCC cytogenetic procedures. The protocol for PCC fusion proved to be effective at doses as high as 20 Gy, enabling the analysis of ring chromosomes and excess PCC fragments. The ring frequencies remained constant during the 8–24-h repair time; the pooled dose relationship between ring frequency (Y) and dose (D) was linear: $Y = (0.088 \pm 0.005) \times D$. During the repair time, excess fragments decreased from 0.91 to 0.59 chromatid pieces per Gy, revealing the importance of information about the exact time of exposure for dose assessment on the basis of fragments. Compared with other cytogenetic assays to estimate radiation dose, the PCC-Rf method has the following benefits: a 48-h culture time is not required, allowing a much faster assessment of dose in comparison with conventional scoring of dicentric and rings in assays for chemically-induced premature chromosome condensation (PCC-Rch), and it allows the analysis of heavily irradiated lymphocytes that are delayed or never reach mitosis, thus avoiding the problem of saturation at high doses. In conclusion, the use of the PCC fusion assay in conjunction with scoring of rings in G0-lymphocytes offers a suitable alternative for fast dose estimation following accidental exposure to high radiation doses.

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1. Introduction

Chromosomal aberrations in peripheral blood lymphocytes are used as biomarkers of exposure to estimate of radiation dose when a nuclear or radiological emergency is investigated. Particularly, rapid dose estimation based on such biomarkers is useful for planning the treatment of highly exposed persons. For this purpose, the analysis of dicentric chromosomes has been for many years, and

still is today, the golden standard and the method most frequently used, because of its specificity and precision. In cases of high-dose radiation exposure, however, there are some disadvantages of the dicentric assay, e.g., underestimation of the dose due to cell death, saturation of dicentrics, and delay in cell-cycle progression due to impaired cell proliferation [1]. The assay is also time-consuming, with 2–3 days being required for lymphocyte culture and scoring.

The drug-induced premature chromosome-condensation (PCC) technique [2] in combination with the scoring of ring chromosomes (PCC-Rch) demonstrated the possibility to avoid saturation of dicentrics after exposure to high doses of low-LET ionizing radiation [3–6]. This method was applied after neutron irradiation [5,7] and proved its usefulness for high-dose estimation after the Tokaimura criticality accident [4]. Moreover, it was a promising approach to reduce scoring time at high radiation doses when the number of rings per cell becomes sufficiently high and the dose estimate may be made with reasonable statistical uncertainties from

Abbreviations: PCC, premature chromosome condensation; PCC-Rch, rings in chemically induced lymphocyte prematurely condensed chromosomes; PCC-Rf, rings in lymphocyte prematurely condensed chromosomes mediated by cell fusion; CHO cells, Chinese hamster ovary cells.

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the analysis of only 50 cells with PCC, which requires just one man-hour [3]. However, the assay requires the same 2–3-day period for lymphocyte culture and chromosome aberration analysis, and inherits, therefore, some of the disadvantages of the conventional dicentric assay.

The direct visualization of chromosome aberrations in non-stimulated G0 lymphocytes by mitotic fusion and induction of premature chromosome condensation (PCC fusion) seems to be an appropriate approach to radically diminish the response time in emergency situations. The PCC fusion method reported by Johnson and Rao in 1970 [8] was improved and simplified with the use of polyethylene glycol (PEG) as a fusogen instead of Sendai virus, and also with the standardization of the fusion conditions, resulting in a simple, rapid and reproducible PCC protocol for G0 lymphocytes in peripheral blood, which cannot be satisfactorily fused by means of Sendai virus [9]. This method does not require the culture of the irradiated lymphocytes, saving thus not only the 48 h of culture time needed by both the conventional cytogenetic method and the chemical-induced PCC assays, but also allowing the analysis of both T and B lymphocytes, thus enabling the use of a larger number of lymphocytes as biodosimeters. The PCC fusion method is particularly advantageous in cases of partial-body exposure to high radiation doses, as it allows the immediate analysis of the remaining irradiated lymphocytes – both T and B cells – in the blood. In contrast, following a 2-day culture period, damaged cells will be delayed in their cell-cycle progression and, as a result, mostly non-irradiated cells will be available for analysis at the first post-irradiation metaphase when the conventional dicentric assay is used. The PCC fusion assay has been used in radiation cytogenetics mainly at low doses to study the repair kinetics of chromosome fragments and the formation of exchanges [10,11]. In addition, several authors have also used this assay for biodosimetry studies, e.g., by scoring excess fragments in Giemsa-stained cells after exposure to high-LET radiation [12], by identifying rings, dicentrics and excess fragments after C-banding [13], or by applying fluorescence in situ hybridization (FISH) for the identification of fragments, translocations and dicentrics [14,15]. The detection of partial-body irradiation after in vivo exposure in a monkey model has also been tested by means of the PCC fusion assay [16].

In the present work, we propose the PCC methodology to estimate exposure to high-dose gamma radiation, in combination with the scoring of rings induced in peripheral blood lymphocytes in G0, at doses of up to 20 Gy. The use of the PCC fusion assay in conjunction with ring scoring in Giemsa-stained G0-lymphocytes offers a suitable alternative method for rapid dose estimation following accidental exposure to high doses of radiation.

2. Materials and methods

2.1. Study design

Four series of experiments were carried out, irradiating either isolated lymphocytes or whole blood, allowing for repair processes of 8 or 24 h for ring formation, in a 5% CO₂ incubator at 37 °C, followed by fusion of isolated lymphocytes with mitotic CHO cells. Chromosome spreads were prepared for scoring ring chromosomes and excess PCC fragments (in excess of 46).

The irradiated samples were identified as follows: (A) isolated lymphocytes with 8 h of repair, (B) isolated lymphocytes with 24 h of repair, (C) total blood with 8 h of repair and (D) total blood with 24 h of repair.

2.2. Blood samples and irradiation

Peripheral blood from six healthy individuals aged between 25 and 45 years, was drawn in heparinized tubes. The blood of the six donors, three females and three males, was assayed as isolated lymphocytes in series A and B, while two females and two males from the six donors were assayed as whole blood in series C and D. Informed consent was obtained from each donor. Total blood or isolated lymphocytes were irradiated in a GammaCell 220 irradiator (Atomic Energy of Canada, Ltd., Ottawa) with 5, 10, 15 and 20 Gy, at room temperature and at a dose rate of about 0.5 Gy/min; dosimetry was performed with a Victoreen r-meter.

2.3. Lymphocyte isolation

Heparinized blood samples were subject to Ficoll-Paque gradient sedimentation to isolate peripheral blood lymphocytes (PBLs) according to the procedures suggested by the manufacturer. The lymphocytes were kept in McCoy's 5A culture medium supplemented with 10% fetal bovine serum (FBS), 1% glutamine and antibiotics (penicillin: 100 U/ml; streptomycin: 100 g/ml). The cells were irradiated (series A and B) or used for cell fusion after total blood irradiation (series C and D).

2.4. Cell culture, cell fusion and PCC induction

Chinese hamster ovary (CHO) cells were grown in McCoy's 5A medium supplemented with 10% fetal calf serum, 1% L-glutamine and antibiotics and incubation at 37 °C in a humidified atmosphere with 5% CO₂. They were maintained as exponentially growing monolayer cultures in 75-cm² plastic flasks at an initial density of 4 × 10⁵ cells per flask. Colcemid at a final concentration of 0.1 g/ml was added to a CHO culture for 4 h and the accumulated mitotic cells were harvested by selective detachment and used as a supply of mitotic cells to induce PCC. The mitotic cells obtained (1.5 × 10⁶/shake/flask) were fixed and checked under the microscope, then kept on ice or frozen until needed for fusion with interphase cells for PCC induction. The mitotic index of the CHO cells was found to be higher than 95% in all experiments.

Cell fusion and induction of PCCs by use of polyethylene glycol (PEG) were performed essentially as described previously [9]. Briefly, mitotic CHO cells and non-stimulated (G0) PBLs were washed separately with serum-free McCoy's 5A medium and mixed at a ratio of about 1:5 in a 15-ml round-bottom culture tube in the presence of colcemid. After centrifugation at 200 × g for 5 min, the supernatant was discarded without disturbing the cell pellet and 0.15 ml of 50% (w/v) polyethylene glycol (PEG 1500, Boehringer Mannheim) was added forcefully and left for about 1 min. Subsequently, 1.5 ml of PBS was slowly added, the tube was shaken gently and the cell suspension was centrifuged at 200 × g for 5 min. The supernatant was discarded and the cell pellet was re-suspended in 0.7 ml pre-warmed McCoy's 5A medium. After 50–60 min at 37 °C, cell fusion and PCC induction were completed. The chromosome spreads were then prepared by standard cytogenetic procedures. Air-dried slides were stained in 3% Giemsa solution.

The PCC fusion index (PCC-f index) was determined as the percentage of interphase cells (lymphocytes) showing prematurely condensed chromosomes [9].

2.5. Scoring process

Scoring of rings and fragmented chromosomes involves the manual identification of the human interphase PCC spreads, which appear as a group of single chromatids, next to the CHO mitotic chromosomes, which can be easily distinguished by morphology and condensation. A representative picture of non-irradiated lymphocyte PCCs demonstrating 46 single chromatid chromosomes is shown in Fig. 1. Excess PCC fragments (more than 46) were scored at each experimental point in at least 50 PCC cells [17,18] on the monitor by use of light microscopy coupled with an image-analysis system (Ikaros MetaSystems, Germany). Rings could also be easily scored on the monitor or directly by microscopy. A circular shaped chromatid with a central opening (see Fig. 2) was scored as a PCC ring. Small circular chromatids with or without central openings were also considered as rings (see short arrows in Fig. 4). For each experimental point at least 100 rings were scored as was suggested for dicentrics [1] in order to give a reasonably accurate dose estimate. The frequency of PCC-Rf was evaluated as the ratio between rings scored and cells analyzed.

2.6. Statistical analysis

Dose-effect relationships were fitted according to a linear model with the aid of DoseEstimate software [19]. The significance of slope (alpha-coefficient) was tested in a t-test and the goodness-of-fit was tested with the Chi-square test. The U-test was used to check whether dispersions of aberrations could be described by a Poisson distribution. The differences between the slopes were tested by means of the F-test, while differences between aberration frequencies were tested with the t-test.

3. Results

The protocol for PCC induction by cell fusion proved to be effective even at doses as high as 20 Gy, giving a sufficient number of PCC spreads with adequate morphology, allowing the analysis of rings and excess PCC fragments (Figs. 1–4). A PCC-f index of 15.5% was obtained in control cells (0 Gy) and after irradiation with 20 Gy.

The yield of PCC-Rf and excess PCC fragments increased with increasing radiation dose (Figs. 2–4).

Table 1 shows the number of PCC cells scored, the frequency of PCC-Rf, the distribution of PCC-Rf by cell with their associated ²/Y- and U-values obtained between 0 and 20 Gy of gamma

Table 1
Frequencies and distribution analysis of PCC rings (PCC-Rf) in four experimental series A–D.

Dose (Gy)	Experimental series	Rings	Cells scored	Frequency rings/cell \pm S.E.	Rings distribution						$^2/y$	U
					0	1	2	3	4	5		
0	^a WB & L ^a	2	300	0.007 ± 0.005	298	2	0	0	0	0	1.00	–0.06
5	A (L 8h ^a)	102	199	0.51 ± 0.051	122	56	17	4	0	0	1.06	0.61
	B (L 24h)	102	161	0.63 ± 0.063	82	61	14	3	1	0	0.94	–0.53
	C (WB 8h)	101	178	0.57 ± 0.056	96	65	15	2	0	0	0.85	–1.39
	D (WB 24h)	94	165	0.57 ± 0.059	101	39	21	3	1	0	1.20	1.85
10	A (L 8h)	119	121	0.98 ± 0.090	37	54	25	5	0	0	0.69	–2.38
	B (L 24h)	102	93	1.10 ± 0.109	31	34	19	7	1	1	1.01	0.08
	C (WB 8h)	102	107	0.95 ± 0.094	43	33	24	7	0	0	0.94	–0.45
	D (WB 24h)	100	79	1.27 ± 0.127	30	17	19	7	6	0	1.27	1.70
15	A (L 8h)	108	79	1.37 ± 0.132	17	26	28	6	2	0	0.72	–1.78
	B (L 24h)	113	76	1.49 ± 0.140	9	31	29	4	3	0	0.56	–2.68
	C (WB 8h)	100	76	1.32 ± 0.132	17	29	21	7	2	0	0.77	–1.39
	D (WB 24h)	112	82	1.37 ± 0.129	20	29	20	10	2	1	0.93	–0.44
20	A (L 8h)	114	64	1.78 ± 0.167	7	13	32	11	1	0	0.47	–2.98
	B (L 24h)	167	92	1.82 ± 0.140	12	24	32	17	7	0	0.69	–2.10
	C (WB 8h)	102	61	1.67 ± 0.166	11	18	15	14	3	0	0.81	–1.04
	D (WB 24h)	114	59	1.93 ± 0.181	5	15	22	13	4	0	0.57	–2.33

^a WB, whole blood; L, lymphocytes; h, hours repair.

radiation in the four experimental series A–D, described in Section 2.1. The distribution of rings among the cells follows a Poisson distribution at most experimental points, although there was a trend toward under-dispersion. This trend was stronger in irradiated isolated lymphocytes than in lymphocytes isolated from irradiated whole blood. The time to score 100 PCC-Rf was about 2 h.

Table 2 shows the frequency of excess PCC fragments/cell obtained between 0 and 20 Gy of gamma radiation for the same experimental series as described for rings. The yield of excess fragments increased with the radiation dose in all experimental series and their distribution showed over-dispersion at all the experimental points. In each experimental series the number of excess fragments increased with dose and decreased as repair time increased from 8 to 24 h. On average, the number of excess fragments/Gy obtained in the 8-h repair experiments (series A and

C) was 0.91 ± 0.2 , and 0.59 ± 0.2 in the 24-h repair experiments (series B and D). No statistically significant differences in background frequency of excess PCC fragments/cell were found in the experimental series, and consequently pooled data on background frequency were used.

Fig. 5 shows the linear dose-response relationships obtained for rings, in the interval 0–20 Gy of gamma radiation. All have a significant slope ($p < 0.05$) and the dose-response is significant, with no differences between experimental series. The relationship obtained with all experimental series pooled was: $Y = (0.088 \pm 0.005) \times D$.

Table 3 shows the coefficients for all experimental series, their slopes and the intercepts with uncertainty for both PCC-Rf and excess PCC fragment relationships, obtained between 0 and 20 Gy of gamma radiation.

4. Discussion

Different strategies have been developed to shorten the response time required in emergency situations after accidental radiation exposure: the triage method reduces the number of



Fig. 1. A representative picture of non-irradiated interphase lymphocytes showing 46 single-chromatid prematurely condensed chromosomes (PCCs). Non-irradiated lymphocytes were incubated for 24 h at 37 °C prior to their fusion to mitotic CHO cells for induction of premature chromosome condensation.

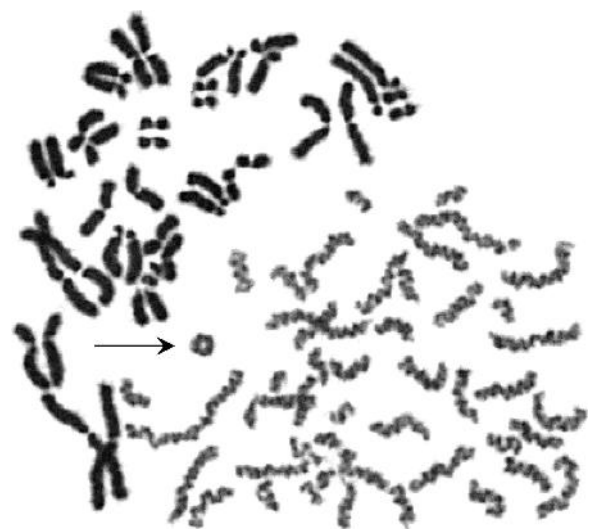


Fig. 2. A circular shaped chromatid in lymphocyte PCCs induced by cell fusion was scored as a ring (PCC-Rf). The arrow shows a PCC-Rf ring formed in a lymphocyte after exposure to 5 Gy followed by a 24-h repair period at 37 °C. In this picture, residual excess PCC fragments (in excess of 46) can also be scored.

Table 2
Frequencies and distribution analysis of excess PCC fragments in four experimental series A–D.

Dose (Gy)	Experimental series	Cells scored	Excess fragments	Frequency frag- ments/cell \pm S.E.	Excess PCC fragments distribution																² /y	U	
					0	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20	21-22	23-24	25-26	27-28	29-30			>30
0	^a WB & L ^a	194	40	0.21 \pm 0.03	173	15	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.26	12.49
5	A (L 8h ^a)	53	127	2.40 \pm 0.21	17	14	11	8	2	1	0	0	0	0	0	0	0	0	0	0	0	2.49	7.64
	B (L 24h)	54	78	1.44 \pm 0.16	31	9	7	5	2	0	0	0	0	0	0	0	0	0	0	0	0	3.26	11.69
	C (WB 8h)	51	138	2.71 \pm 0.23	17	9	12	7	5	1	0	0	0	0	0	0	0	0	0	0	0	2.65	8.28
	D (WB 24h)	51	57	1.12 \pm 0.15	31	13	2	3	2	0	0	0	0	0	0	0	0	0	0	0	0	3.21	11.14
10	A (L 8h)	49	439	8.96 \pm 0.43	6	3	6	4	6	5	5	6	2	2	1	0	1	2	0	0	0	5.03	19.77
	B (L 24h)	51	272	5.33 \pm 0.32	10	6	6	4	15	6	3	1	0	0	0	0	0	0	0	0	0	2.94	9.71
	C (WB 8h)	51	490	9.61 \pm 0.43	1	1	7	5	11	5	5	7	4	2	2	1	0	0	0	0	0	2.52	7.62
	D (WB 24h)	39	262	6.72 \pm 0.42	5	3	2	14	6	4	1	2	0	1	0	0	0	0	0	0	0	2.51	6.60
15	A (L 8h)	56	679	12.13 \pm 0.47	4	2	0	6	3	6	7	4	11	6	1	3	2	1	0	0	0	3.39	12.54
	B (L 24h)	53	564	10.64 \pm 0.45	2	5	4	7	3	9	6	6	3	0	3	1	0	1	1	0	2	4.82	19.47
	C (WB 8h)	51	729	14.29 \pm 0.53	0	0	0	4	4	5	9	10	4	4	2	4	2	0	0	0	1	2.06	5.31
	D (WB 24h)	50	546	10.92 \pm 0.47	3	4	1	2	4	9	9	7	5	1	1	1	1	1	1	0	0	3.09	10.33
20	A (L 8h)	51	1193	23.39 \pm 0.68	0	1	0	1	1	1	3	1	2	3	4	3	5	6	1	8	9	3.04	10.19
	B (L 24h)	51	786	15.41 \pm 0.55	0	1	0	1	2	5	1	6	5	6	9	3	3	1	0	0	1	2.42	7.10
	C (WB 8h)	51	1231	24.14 \pm 0.69	0	0	0	0	0	2	0	2	2	7	6	4	5	3	0	7	10	2.17	5.85
	D (WB 24h)	84	1182	14.07 \pm 0.41	1	1	3	8	7	10	12	9	3	9	3	4	3	5	1	3	1	3.57	16.55

^a WB, whole blood; L, lymphocytes; h, hours repair.



Fig. 3. The yields of PCC-Rf rings and residual excess PCC fragments increase with increasing radiation dose. Arrows point to PCC-Rf rings formed in a lymphocyte irradiated with 10 Gy and analyzed after a 24-h repair period at 37 °C. Eight residual excess PCC fragments can also be easily scored.

metaphases or dicentrics to score [20]; the networks propose sharing the scoring process among different laboratories [1,21,22]; the automation of processing and scoring allows the time required for the scoring process to be reduced [23]; the quick scan with less restrictive scoring criteria, recording dicentrics without the requirement of ensuring the presence of 46 centromeres [24]. These methods, when applied in conventional cytogenetics, diminish the response time needed to obtain dose estimates and they can certainly play an important role in large-scale events, where biomarkers are used to support the clinical decisions, as they provide additional data expected to contribute to the differentiation of symptoms due to radiation exposure. Nevertheless, all the assays described above can only be applied after the 48-h time interval required for T-lymphocyte stimulation. The PCC in combination with FISH painting of chromosome 1 [25] is one option to avoid a prolonged cell-culture time. The improvement of this method by use of only a 6-h culture period followed by Giemsa

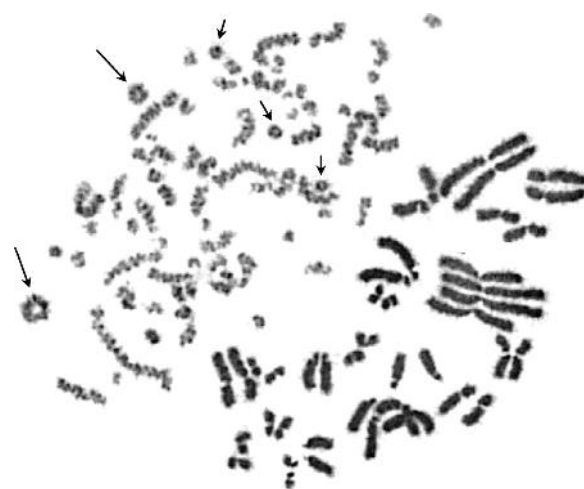


Fig. 4. At very high doses the yield of small PCC-Rf rings, with or without central opening, increases. Short arrows point to 3 small rings formed in a lymphocyte after a dose of 20 Gy and an 8-h repair time at 37 °C. From the analysis of residual excess PCC fragments, 24 fragments in excess of 46 can also be observed.

Table 3

Dose calibration coefficients for PCC-Rf and PCC excess fragments of relationships ($Y=C+D$) obtained between 0 and 20 Gy of gamma radiation in four experimental series A, B, C and D.

Experimental series	Coefficients of relationships			
	PCC-Rf		PCC-excess fragments	
	$a \pm \text{S.E.}$	$C \pm \text{S.E.}$	$a \pm \text{S.E.}$	$C \pm \text{S.E.}$
A (L ^a 8h ^a)	0.088 ± 0.003	0.007 ± 0.005	1.11 ± 0.17	0.21 ± 0.03
B (L 24 h)	0.087 ± 0.007	0.007 ± 0.005	0.78 ± 0.005	0.21 ± 0.03
C (WB ^a 8 h)	0.095 ± 0.009	0.007 ± 0.005	1.26 ± 0.13	0.21 ± 0.03
D (WB 24 h)	0.080 ± 0.005	0.007 ± 0.005	0.96 ± 0.15	0.21 ± 0.03
Pooled data	0.088 ± 0.005	0.007 ± 0.005	–	–

^a WB, whole blood; L, lymphocytes; h, hours repair.

staining was also reported. However, the morphology of the condensed chromosomes obtained after this short-term culture was not suitable for conventional chromosome aberration analysis [26].

In this paper we extend the use of the PCC-fusion assay to the high-dose region of radiation exposure through analysis of ring chromosomes. The PCC-fusion assay has been shown to be a powerful cytogenetic tool [27]; it has been recently used to elucidate the mechanisms underlying conversion of DNA damage into chromosomal damage [18,28]. Our results show that this methodology offers also a suitable alternative for rapid and accurate dose estimation after accidental over-exposure to high doses of radiation. This procedure combines the practically instantaneous visualization of PCCs in interphase lymphocytes after the collection of blood samples from exposed individuals, with the relatively easy and rapid scoring of rings. Previous experience with the PCC-fusion assay was limited to lower-dose intervals up to around 8 Gy [10–14,17].

The scoring of rings for biological dosimetry purposes in chemically induced prematurely condensed chromosomes following culture of the lymphocytes was proposed by Kanda et al. [3], and several dose–response curves for PCC rings (PCC-Rch) in the dose range up to 20 Gy of low-LET radiation have been reported [5,6]. The results obtained in the present work show that the PCC-fusion protocol can also be successfully applied in conjunction with the scoring of rings, in the same dose interval reported for PCC-Rch. In addition, this technique has the advantage of eliminating the two-day culture period that is required for the PCC-Rch and the conventional dicentric assay.

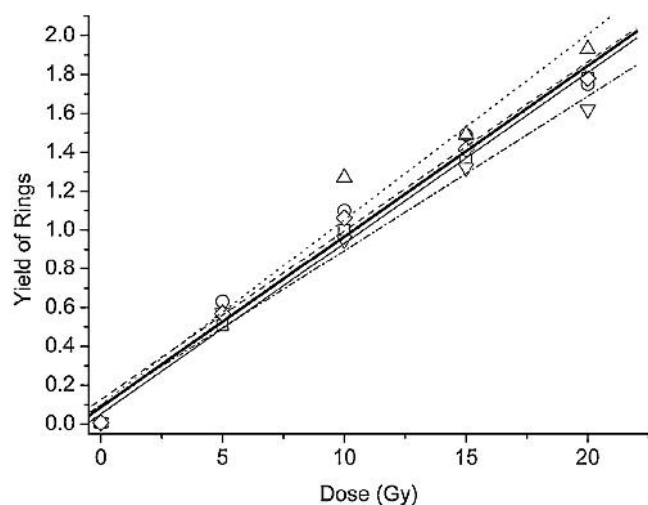


Fig. 5. Dose–response relationships for PCC-Rf frequencies between 0 and 20 Gy of gamma radiation for different experimental series: lymphocytes 8 h repair (○) solid line; lymphocytes 24 h repair (○) dash line; whole blood 8 h repair (○) dash-dot line; whole blood 24 h repair (○) dot line; pooled data (○) solid bold lines.

The scoring of rings after Giemsa staining in the PCC-fusion assay introduces several advantages in comparison with the scoring of excess PCC fragments frequently tested for biological dosimetry purposes.

The first is that the complexity related with the scoring of excess PCC fragments, of which the frequency is particularly elevated after high radiation doses (see Table 2) is significantly reduced. The rings are easily recognized, their frequency is lower but still sufficient for adequate statistical accuracy, and consequently the scoring is a rapid process. Two hours are required on average for the analysis of an appropriate number of rings in PCC in lymphocytes, similar to the scoring time required for PCC-Rch [3,6]. The second important advantage is that the kinetics problem associated with excess fragments declining with time, as reported before [10,11] and also observed here, is solved since the formation of rings remains constant after an 8-h incubation time in the two experimental conditions tested, i.e. isolated lymphocytes or whole blood. Previous results at low doses showed that the PCC-Rf frequencies increased with a repair time up to 6 h and remained constant after 6 or 7 h post-irradiation [11], while Durante et al. – based on earlier reports – considered that in an 8-h incubation period the formation of chromosome exchanges in human lymphocytes is complete [14]. The third, but not less important advantage is the ring distribution in the cells. PCC-Rf among the cells followed a Poisson distribution in most of the experimental points, as expected after a uniform whole-body exposure. This is consistent with previous findings in the low-dose region [11], introducing the possibility to identify partial-body irradiation by use of the tools already reported for conventional cytogenetic analysis, i.e. the U test [1].

The distribution of excess PCC fragments per cell is over-dispersed and, consequently, the identification of partial-body radiation exposure requires the identification of the fraction of damaged cells by the scoring of excess PCC fragments. The fraction of damaged cells and the fraction of the irradiated body should correlate at doses higher than around 3 Gy, when all irradiated cells are damaged [16,17], and considering that the efficiency of the PCC-fusion assay on damaged and non-damaged cells is identical. The apparently constant efficiency of the PCC-fusion assay on damaged cells – suggested on the basis of partial-body simulation studies [17] – was confirmed here with the results obtained with the PCC-f index at doses up to 20 Gy. This index was the same (15%) at 0 and 20 Gy, allowing the analysis of the heavily damaged and un-damaged cells with equal probability.

The frequencies of PCC-Rf obtained in the present work are 4 times higher than those previously obtained by the same team, using the PCC-Rch assay, in the same dose interval and with the same scoring criteria [5]. The PCC-Rf obtained here was also 2 to 3 times higher than the PCC-Rch frequencies reported by other authors for similar dose intervals, using different PCC-Rch protocols and scoring criteria [29]. The background frequency obtained by us for PCC-Rf (0.007) is higher than the background frequency

reported for PCC-Rch (0.002) [6]. Both values are considerably higher than the ring frequency reported for the conventional cytogenetic analysis (0.0002 ring per metaphases) [30]. Additional experimental data that focus on the mechanism of ring formation can clarify the differences in background and radiation-induced frequencies reported using different cytogenetic methods. The selective cell death of highly damaged cells during the culture process [31], the level of maturation-promoting factors in different cell-cycle phases [32,33], and other factors are probably associated with these differences. Nevertheless, for practical application of the PCC-Rf assay, an important aspect is the dose-response relationship obtained after in vitro irradiation [1].

The dose-response relationships from 0 to 20 Gy is better adjusted to a linear model, while in the dose interval from 0 to 8 Gy, covered in previous publications, a linear-quadratic model provided a better fit [11]. This is not surprising, as it is known that in the high-dose region, usually without experimental points at doses below 5 Gy, the dose-response relationship is better adjusted to a linear model [5,6]. On the contrary, the inclusion of experimental points at low doses obstructs the linear adjustment or gives similar results for both the linear and linear-quadratic adjustments [6]. However, in the high-dose region where the quadratic term of the calibration curve is not relevant, the use of a linear model appears to be appropriate.

Finally, is important to remark that the PCC-fusion assay is actually applied in only a limited number of laboratories around the world. Its applicability for biological dosimetry in an emergency response to radiation exposure should be preceded by assimilation of the assay. The detailed explanation of the method is presented in the last IAEA manual on cytogenetic dosimetry [1]. A sufficient number of mitotic inducer cells ready for the application of the assay can be obtained by maintaining a CHO culture in the laboratory, or by using frozen CHO cells [1,13].

5. Conclusions

Our results show that the PCC-Rf assay is appropriate for use as a biodosimeter after whole-body exposure to high doses of ionizing radiation. The assay covers a wide dose range and the results can be obtained, with appropriate uncertainties, shortly after the reception of blood samples from exposed individuals, without need to culture the lymphocytes.

It should be recognized that although automation and image analysis enables quick scoring of chromosomal aberrations, the PCC-Rf assay is performed directly under the microscope and offers a rapid, easy and accurate technique for dose assessment.

Conflict of interests statement

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of the paper.

Authors' contribution

Conceived the experiments: OG and GP; Designed the experiments: AIL, OG and GIT; Performed the experiments and scoring: AIL, GIT, GP and IR. Analyzed the data: AIL, JEG and OG; Wrote the paper: AIL and OG; Reviewed the manuscript: GP.

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