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

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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 ⁶⁰Co 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 D₀ 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; D₀ 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 D0 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, 0.5, 1, 5, 7.5, 10, 15 and 20 Gy (dose-rate 0.5 Gy/min) using a source of 60Co 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 D0 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 1 h 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 (σ²/Y) was analyzed for each dose and each proportion of irradiated blood, assuming Poisson distribution if u ≤ 1.96, and overdispersion if u > 1.96.

The percentage of correct SPBI identifications (%SPBI\(_D\)) was calculated as follows:

\[
\%SPBI_D = \frac{\#SPBI_{u>1.96}}{\#SPBI} \cdot 100,
\]

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.

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<th>2</th>
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<th>4</th>
<th>5</th>
<th>Y ± SE</th>
<th>σ²/Y</th>
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Continued
D0 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 D0 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 (%Fcorrect) was calculated as follows:

\[ %F_{\text{correct}} = \frac{\#F_{10\%}}{\#F_T} \times 100, \]

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 (%Dcorrect–SPBI) was calculated as follows:

\[ %D_{\text{correct–SPBI}} = \frac{\#D_{\text{SPBI–30\%}}}{\#D_{\text{SPBI–T}}} \times 100, \]

where \( \#D_{\text{SPBI–30\%}} \) is the number of estimated SPBI doses within 30% of the true dose, and \( \#D_{\text{SPBI–T}} \) is the total number of estimated SPBI doses.

Table 1. Continued

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<th>Irradiated Blood</th>
<th>Dose (Gy)</th>
<th>Donor</th>
<th>Cells</th>
<th>Rings</th>
<th>Distribution of rings</th>
<th>Y ± SE</th>
<th>σ²/Y</th>
<th>u</th>
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<td>170</td>
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<td>0.675 ± 0.055</td>
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<td>104</td>
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<td>100%</td>
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<td>510</td>
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<td>503</td>
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<td>0.014 ± 0.005</td>
<td>0.99</td>
<td>-0.20</td>
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<td>500</td>
<td>8</td>
<td>492</td>
<td>8 0 0 0 0</td>
<td>0.016 ± 0.011</td>
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<td>-0.24</td>
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<td>0.016 ± 0.009</td>
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<tr>
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<td>0.380 ± 0.003</td>
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<td>300</td>
<td>114</td>
<td>210</td>
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<td>0.380 ± 0.005</td>
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<td>1.63</td>
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<tr>
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<td>102</td>
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<td>1.000 ± 0.018</td>
<td>1.07</td>
<td>0.49</td>
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<td>108</td>
<td>75</td>
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<td>0.720 ± 0.012</td>
<td>1.03</td>
<td>0.24</td>
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<td>101</td>
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<td>0.660 ± 0.011</td>
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<td>0.733 ± 0.017</td>
<td>1.02</td>
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<td>107</td>
<td>66</td>
<td>36 21 8 0 1</td>
<td>0.811 ± 0.018</td>
<td>1.23</td>
<td>1.84</td>
</tr>
</tbody>
</table>

The u value was used to assess the partial irradiation.
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 ($\sigma^2/Y$) 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 1](https://academic.oup.com/jrr/article-abstract/54/5/863/1000275)
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.

**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
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.

**D₀ value**

Here we present the first attempt to calculate a D₀ 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 D₀ values reported for the dicentric assay for X-rays (D₀ = 2.7 [15], D₀ = 3.8 [19]) or gamma radiation (D₀ = 3.5 [16], D₀ = 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 D₀ is based on the ability of G₀-irradiated lymphocytes to reach G2-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.

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 D₀ 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.

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**REFERENCES**


