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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 γ-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 dicentrics 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.

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 dicentrics 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%-CO_2 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 μg/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% CO_2. They were maintained as exponentially growing monolayer cultures in 75-cm^2 plastic flasks at an initial density of 4×10^5 cells per flask. Colcemid at a final concentration of 0.1 μg/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 (1.5×10^6/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 $200 \times g$ 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 $200 \times g$ 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 σ^2/Y- and *U*-values obtained between 0 and 20 Gy of gamma