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• ***Short report***

Construction of a dose – response curve by induction of premature chromosome condensation for biological dosimetry

A. Mahmoudzadeh Emamchai [1*], H. Mozdarani[2], S. Mohammadifrad[1]

[1] Novin Medical Radiation Institute, Tehran, Iran
[2] Department of Medical Genetics, School of Medical Science, Tarbiat Modarres University, Tehran, Iran

Premature Chromosome Condensation (PCC) appears to have a possible utility for biological dosimetry purposes. The PCC technique may be adapted for cases of suspicion of overexposure where sampling is performed at least one day after an accident. For this purpose, human blood samples were exposed *in vitro* to ^{60}Co up to 10 Gy and the PCC technique was performed immediately after irradiation. Analysis of excess PCC fragments distribution showed an over dispersion and the dose- effect relationship was best characterized by linear regression. **Iran. J. Radiat. Res., 2009; 6 (4): 213-218**

Keywords: *Gamma rays, premature chromosome condensation, biological dosimetry, dose response curve.*

INTRODUCTION

The induction of chromosomal aberration is one of the several biological responses to ionizing radiation which have been investigated as a means of estimating an individual's average whole body dose. Biological dosimetry based on dicentric or micronuclei scoring in peripheral blood lymphocytes after *in vitro* stimulation have the limitation of the low number of lymphocytes present in the blood after higher doses of ionizing radiation or experiencing mitotic delay [1-3]. Thus, the scored mitoses might not be representative of the exposed cell population. These limitations have been overcome with the advent of premature chromosome condensation (PCC), first reported by Johnson and Rao [4]. This technique is considered as a potent biodosimetric tool [5], since it is the most sensitive method for analyzing the initial chromosome damage after irradiation [6]. The PCC assay is useful to determine the exposure to low dose as well as following a life-threatening high acute dose of low and high LET ionizing radiation. Moreover, it can discriminate accurately between total and partial body exposure [5]. However, this procedure is technically difficult and the PCC index is generally low and unstable [7, 8]. The construction of the dose-response curves with this method [9-12] indicates that PCC induction is a powerful method for biodosimetry in the case of a very high dose irradiation. In this paper we report the dose response curve constructed based on chromosomal aberration scoring in PCC induced human peripheral lymphocytes.

MATERIALS AND METHODS

Cell culture and mitotic harvest

The Chinese hamster ovary (CHO) cell line, (A gift from Prof. F. Darroudi, Leiden University), was used as mitotic inducer for PCC assay. CHO cells were cultured in complete growth medium consisting of RPMI-1640 (Gibco-BRL) supplemented with 10% fetal calf serum (Gibco) and 1% penicillin- streptomycin (Gibco-BRL). Cells were grown in 75cm^2 tissue culture flasks (Falcon) at 37 °C in a humid atmosphere of

**Corresponding author:*
Mr. Aziz Mahmoudzadeh Emamchai, Novin Medical Radiation Institute, Tehran, Iran.
Fax: *+98 21 88086782*
E-mail: *mehdi_53@email.com*

5% CO_2, and were sub-cultured twice a week. Mitotic CHO cells were harvested by the mitotic shake-off procedure 4-5 hours after adding 0.1 µg/ml colcemid (Gibco-BRL). Harvested mitotic cells were stored at -110 °C for later use.

Sample collection and lymphocyte isolation

Blood samples (about 10 ml) were collected by venipuncture in heparinized tubes from four healthy male donors (mean age 25 ± 3). The study was approved by the Ethical Committee of the Novin Medical Radiation Institute. All donors gave their informed written consent and completed a written questionnaire to obtain information related to their lifestyle, such as dietary habits, medical history and exposure to chemical and physical agents. Lymphocytes were isolated from whole peripheral blood by centrifuge at 3000 rpm for 30 minutes at 18°C using ficoll- hypaque (Baharafshan, Iran). Isolated cells were washed three times with RPMI-1640 and counted using hemocytometer.

Irradiation

Lymphocytes were suspended in cryo-tubes with 2 ml complete RPMI-1640 medium supplemented with 10% fetal calf serum. Samples were gamma irradiated using a ^{60}Co source (Theratron II-780C, Canada) in a 37 °C water bath, at doses of 0, 0.5, 1, 2, 3, 4, 5 and 10 Gy at a dose rate of 109.23 cGy/min at room temperature (23 ± 2 °C).

Induction of PCC (premature chromosome condensation)

PCC was induced according to the procedure of IAEA [13]. Before fusion, CHO mitotic cells were thawed to defreeze with pre-warmed RPMI-1640 medium (10 ml). The cells were centrifuged for 1000 rpm/ 7 min twice. Then about 1×10^6 mitotic cells were mixed with 5×10^6 G_0 lymphocytes in a round- bottomed culture tube and washed again with RPMI-1640. After centrifugation (1000 rpm, 7 min), the supernatant was discarded. PEG (150 µl, 40% w/v, MW 1450, Sigma) was added onto the cell pellet, and left in place without shaking. After 1.5 minute, 2.5 ml RPMI (without FCS) was added to dilute PEG and cells were washed twice and supernatant was removed. Subsequently 760 µl RPMI and 35 µl colcemid was added to the tube and incubated 1 h at 37°C for chromatin condensation. Cells were harvested and exposed to 8-10 ml pre-warmed hypotonic (0.075 M/L) solution for 20 minute at 37 °C then fixed in Carnoy's fixative, methanol: acetic acid (3:1, V/V). Slides were prepared using air drying technique and stained in 10% Giemsa solution for 5 minutes. Hybrid cells were observed under a bright- light microscope at ×1000 magnification. The number of chromosome fragments were counted in each cell (in untreated samples 2n= 46), and extra chromosomal breaks above 46 were considered as chromosome damage induced by radiation (figure 1).

RESULTS AND DISCUSSION

Frequency and distributions of PCCs and fragments in lymphocytes exposed to representative doses of gamma radiation are shown in table 1. Analysis of the yield of PCCs and fragments including determination of the mean ± 2SD (standard deviation) (figure 2) and the p value (sig 0.035) for evaluation of the frequency of distribution (Poisson or over dispersion) was done by the use of one-sample Kolmogrov - Smirnov test, at 95% confidence level. Test distribution was normal too. One way ANOVA test showed increasing of chromosome fragments with increasing radiation dose ($p < 0.001$). The dose- effect relationship was found in this study using pooled data from 4 experiments. Dose – response curve for the number of excess PCC fragments were fitted to straight lines by the weighted least- squares regression method ($Y= 44.71+ 4.78\ x$) (figure 3).

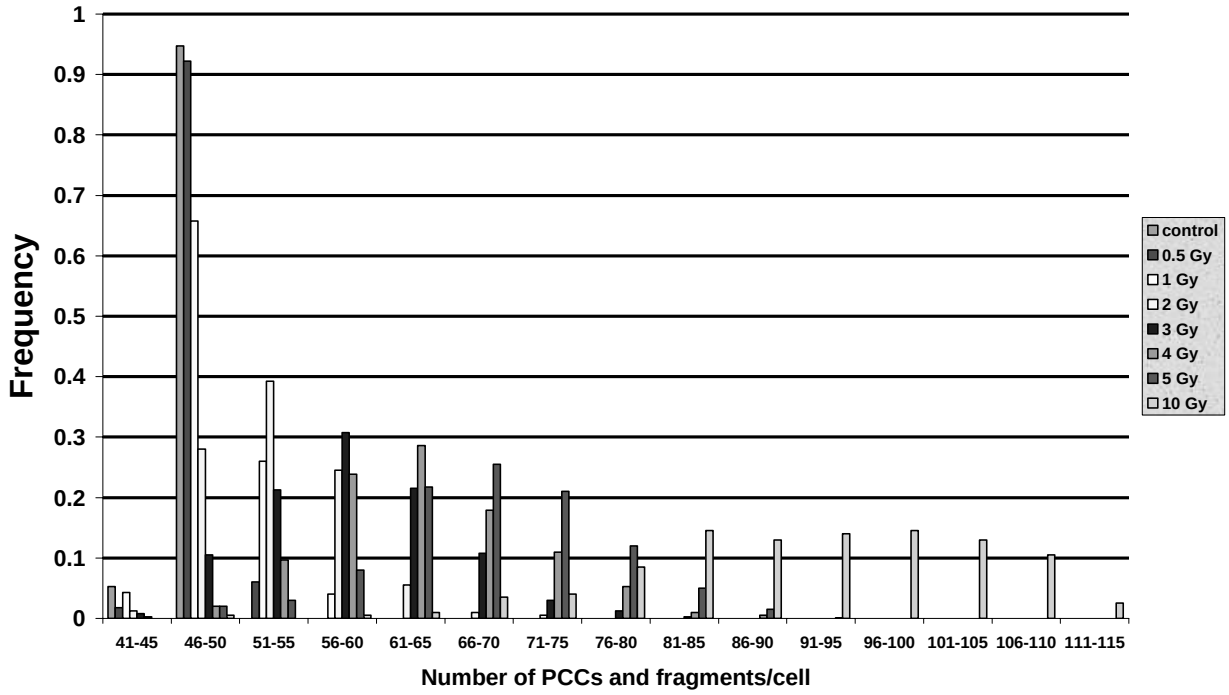

Figure 1. The number of frequency of PCCs and fragments/cell in different doses (0-10 Gy).

Table 1. Distribution analysis of gamma- ray dose response data.

Dose (Gy)	Number of cells	PCCs and Fragments/ Mean ± SD
0	400	46
0.5	400	47.13±0.058
1	400	49±0.042
2	400	53.4±00.90
3	400	58.78±0.32
4	400	63.73±1.12
5	400	64.64±1.225
10	200	92.95±1.06

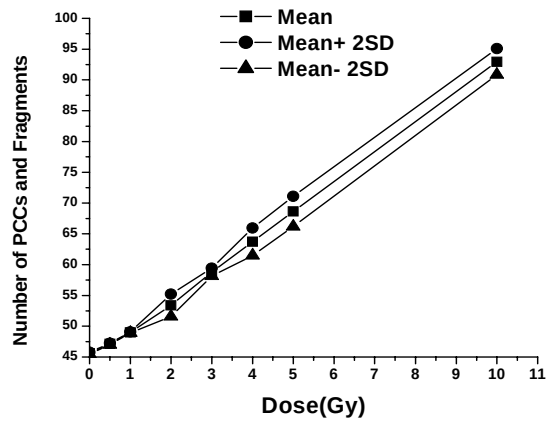

Figure 2. Dose response relationship for the yield of excess PCC fragments immediately after radiation.

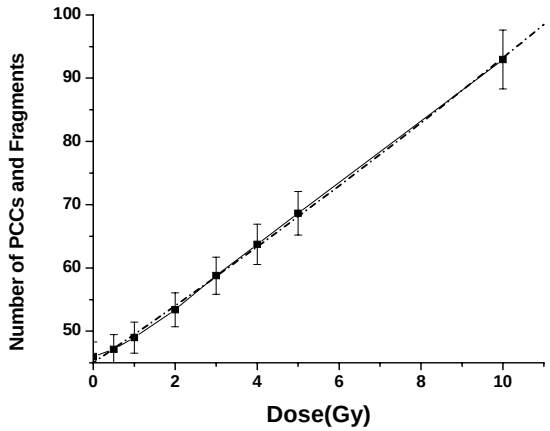

Figure 3. Excess PCC fragments were fitted to straight lines by the weighted least- squares regression.

The present study report the first dose – effect relationships established in the human lymphocytes in Iran using the technique of PCC, similar to as described by other investigators in other countries [14-16]. The linear dose effect relationship observed in the dose range of 0-10 Gy in our study, in general, is in agreement with previously published dose response relationship with the same method [17,18]. Over dispersion was observed in our results are also in agreement with the findings of other investigators [5, 19, 20]. It is generally assumed that dicentric and centric ring distribution follows a Poisson law, but over dispersion at low energy [21]. Another explanation for this over dispersion could be the random scoring error as PCC samples were considerably more difficult to analyze than metaphase spreads [21]. Finally this study reports a specific adaptation of the PCC method for biological dosimetry. In comparison with conventional cytogenetic technique, the potential advantage of PCC assay is to overcome the culture step and also to eliminate the problem of mitotic delay and interphase death leading to an underestimation of the received dose. In conclusion, the premature chromosome condensation technique could be applied to an accident situation.

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