Mutation Research 807 (2016) 47–54

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Dose response of multiple parameters for calyculin A-induced premature chromosome condensation in human peripheral blood lymphocytes exposed to high doses of cobalt-60 gamma-rays

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ARTICLE INFO

Article history:
Received 6 January 2016
Received in revised form 16 June 2016
Accepted 29 June 2016
Available online 27 July 2016

Keywords:
Ionizing radiation
Premature chromosome condensation
Dose response
Multiple parameters

ABSTRACT

Many studies have investigated exposure biomarkers for high dose radiation. However, no systematic study on which biomarkers can be used in dose estimation through premature chromosome condensation (PCC) analysis has been conducted. The present study aims to screen the high-dose radiation exposure indicator in calyculin A-induced PCC. The dose response of multiple biological endpoints, including G_2/A-PCC (G_2/M and M/A-PCC) index, PCC ring (PCC-R), ratio of the longest/shortest length (L/L ratio), and length and width ratio of the longest chromosome (L/B ratio), were investigated in calyculin A-induced G_2/A-PCC spreads in human peripheral blood lymphocytes exposed to 0–20 Gy (dose–rate of 1 Gy/min) cobalt-60 gamma-rays. The G_2/A-PCC index was decreased with enhanced absorbed doses of 4–20 Gy gamma-rays. The G_2/A PCC-R at 0–12 Gy gamma-rays conformed to Poisson distribution. Three types of PCC-R were scored according to their shape and their solidity or hollowness. The frequencies of hollow PCC-R and PCC-R including or excluding solid ring in G_2/A-PCC spreads were enhanced with increased doses. The length and width of the longest chromosome, as well as the length of the shortest chromosome in each G_2/M-PCC or M/A-PCC spread, were measured. All L/L or L/B ratios in G_2/M-PCC or M/A-PCC spread increased with enhanced doses. A blind test with two new irradiated doses was conducted to validate which biomarker could be used in dose estimation. Results showed that hollow PCC-R and PCC-R including solid ring can be utilized for accurate dose estimation, and that hollow PCC-R was optimal for practical application.

1. Introduction

Biological biodosimetry, which uses the analysis of dicentrics or micronuclei in human peripheral blood lymphocytes, is a routine, precise dose estimation method after accidental radiation exposure, especially for acute radiation exposure to doses of below 5 Gy gamma-rays or X-rays [1–3]. However, problems, such as radiation-induced mitotic delay and cell death, especially after high doses (i.e., over 10 Gy whole-body radiation), can underestimate of the radiation exposure dose using these methods. As a result, the dicentric or micronucleus analysis will be difficult or even impossible in such cases. The premature chromosome condensation (PCC) method, which uses cell fusion or chemical inhibitors of protein phosphatases (okadaic acid or calyculin A), offers a potential means to overcome the problem of mitotic delay or cell death after exposing to high doses of radiation [4–9]. This method enables the condensation and visualization of interphase chromatin similar to mitotic chromosomes, thus enabling chromosome analysis in metaphase chromosomes and interphase chromatin.

The PCC induction through the fusion of human lymphocytes with mitotic Chinese hamster ovary cells in the presence of polyethylene glycol or Sendai virus enables the chromosomes in the G_1, S or G_2 stage to condense prematurely immediately after irradiation without the need for any mitogen stimulation and culturing [4]. This assay can be used to detect exposure to low doses and to life-threatening high acute doses of ionizing radiation. The hardest point of this assay is the cell fusion technique, which requires the maintenance the Chinese hamster ovary cells in the exponential growth phase. However, induction frequency is relatively low [4–6]. Given these drawbacks, the cell fusion PCC method can only

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http://dx.doi.org/10.1016/j.mrgentox.2016.06.010
1383-5718/

be conducted in a few laboratories. The chemically induced PCC, with inhibitors of DNA phosphorylation such as okadaic acid or calyculin A, can be easily performed in every cytogenetic laboratory [10]. Calyculin A can induce PCC about 20 times more effectively than the okadaic acid [8].

For the calyculin A-induced PCC technique, 48 h cell culture time and calyculin A is added to the culture during the final hour. Using chemically induced PCC, several biological endpoints can be analyzed, such as excess fragments [11–13], PCC rings (PCC-R) [13,14], and dicentric or total chromosomal aberration [15,16]. The excess fragment assay is not used extensively because the number of induced fragments at high doses is too large for reliable visual analysis. The dicentric analysis difficult to complete because of the poor chromosome morphology in Giemsa-stained PCC spreads. The PCC-R is the most popular endpoint in chemically induced PCC analysis used in biological dosimetry. Recently, the ratio of the longest/shortest length of Giemsa-stained chemically induced PCC (L/L) [17,18] or cell-cycle progression index [19] has been found to be a potential indicator of radiation exposure. No systematic study on which biomarker can be used in dose estimation has been observed through PCC analysis.

The present study attempted to investigate the dose response of multiple biological endpoints, including G_2/A-PCC (G_2/M-PCC and M/A-PCC) index, PCC-R, L/L ratio and length and width ratio of the longest chromosome (L/B), in calyculin A-induced G_2/A-PCC spreads in lymphocytes exposed to 0–20 Gy cobalt-60 gamma-rays. This study aimed to systematically compare the potential high-dose radiation exposure indicators on the same PCC specimen to screen the optimal biomarker of high-dose radiation exposure. A blind test using irradiated human blood samples exposed to two doses of cobalt-60 gamma-rays was performed to compare indicators and to identify the optimal one for dose estimation.

2. Materials and methods

2.1. Subjects and blood collection

The study plan and the informed consent were approved by the ethics committee of the National Institute for Radiological Protection, Chinese Center for Disease Control and Prevention.

Human peripheral blood samples were collected from three healthy male donors for the dose–response relationship. Subjects were 26, 28, and 43 years old. Human peripheral blood samples from one male (25 years old) were collected for the blind test. All donors had no history of smoking, chronic disease, substance abuse, or toxic chemical exposure. Moreover, they had no radiation exposure or viral infection six months preceding the study. The study scope was explained to each subject, and written informed consent was obtained.

Once the informed consent was obtained, whole peripheral blood samples were collected by venipuncture into vacutainers containing lithium heparin as an anticoagulant (BD Biosciences, Franklin Lakes, USA).

2.2. Sample irradiation

Human peripheral blood samples were divided into five aliquots, which were contained in 15 ml centrifuge tubes, and irradiated with 0 (sham irradiation), 4.0, 8.0, 12.0, 16.0 or 20.0 Gy, respectively, at a dose rate of 1.0 Gy/min at 37 °C using a cobalt-60 gamma-ray source provided by the Beijing Radiation Center, Beijing Normal University. The source radioactivity was 130 TBq, and the homogeneous irradiation field was 30 cm × 30 cm. The exposure setup was calibrated through physical measurement using an ionizing chamber. Calibration uncertainty was 1.0%. The human blood samples in 15 ml centrifuge tubes were placed into a 37 °C water bath during irradiation to permit electronic equilibrium. Irradiated whole blood samples were maintained at 37 °C for 2 h for DNA repair.

Human peripheral blood sample was divided into two aliquots for the blind test. The irradiated doses were 5.5 and 10.0 Gy each. The irradiation condition was the same as above.

2.3. Cell culture and slide preparation

Approximately 0.5 ml peripheral blood was cultured in 4.5 ml fresh RPMI-1640 culture medium (Invitrogen, Carlsbad, USA) containing 0.2 mg/ml phytohemagglutinin (Invitrogen, Carlsbad, USA), antibiotics and 20% fetal calf serum (HyClone, USA). The mixtures were incubated at 37 °C for 48 h. Calyculin A (Sigma, USA) was added to the 50 nM final concentration for 2 h before harvest. The cells were then collected and treated with a hypotonic solution (0.075 M KCl) for 10 min at 37 °C, followed by two rounds of fresh fixation with methanol–acetic acid (3:1, v/v). A few drops of the cell suspension in fixative were dropped onto dried, alcohol precleaned slides. Afterwards, the slides were placed above a container filled with heated water and then prepared in ice-cold humid conditions and stained with Giemsa. For the blind test, the slides were coded with random numbers and not disclosed until the dose estimation was completed to avoid selective bias from the scorer.

2.4. PCC image capture and scoring criteria

A total of 1000 cells were observed for each dose level to obtain the G_2/A-PCC index. The cells were divided into non-PCC cells (non-transformed and dead cells), as well as G_1-PCC, S-PCC, G_2/M-PCC, and M/A-PCC cells. The G_2/A-PCC index refers to the ratio of G_2/A-PCC (G_2/M-PCC and M/A-PCC) cells to the 100 cells analyzed.

The PCC-R was scored only in the G_2/A-PCC spreads because the chromosomes in the G_1-PCC and S-PCC spread were unclear and indistinguishable. G_2/M-PCC spreads shows the sister chromatids, and M/A-PCC cells shows separated chromatids (Fig. 1). For the G_2/A-PCC, the spreads were viewed with a Zeiss Axioplan 2 Imaging microscope (Zeiss, Oberkochen, Germany) with a cooled charge-coupled device (AxioCamHRM, Zeiss) and the black-and-white images were captured and stored. About 200–400 G_2/A PCC cells were analyzed for each subject at each dose level.

Three types of PCC-Rs were observed in the present study: hollow, kidney-shaped and solid rings (Fig. 1). The criteria for these three kinds of PCC-R are as follows. A hollow PCC-R has one or two hollow circular structures. The two hollow PCC-Rs in the G_2/M phase may be part or complete overlapping, or they may have separated for a certain distance to become a pair of rings. A kidney-shaped PCC-R is usually present in the G_2/M phase, and two rings are twisted or folded into a pair of kidney-shaped structure. A solid PCC-R has one or two spheroid structures that may be more compact than normal chromosomes. However, its diameter is not less than the width of a chromatid.

Five different forms of PCC-Rs were recorded based on the morphological characteristic (Fig. 1): single hollow PCC-R, double hollow PCC-R, kidney-shaped PCC-R, double solid PCC-R, and single solid PCC-R. As the morphology of a solid PCC-R is extremely difficult to distinguish from that of the small chromosome or a chromatid fragment, the observed frequency of the solid ring from same slides varies among different scorers. Thus, the total PCC-R was calculated including or excluding solid rings in this study.