Chromosome Aberrations in Human Lymphocytes Irradiated with Ionizing Radiation

Tae Ho Ryu , Jin Hong Kim , Jin Kyu Kim *
Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute,
29 Geumgu-gil, Jeongup, 580-185, Korea
**Corresponding author: jkkim@kaeri.re.kr*

1. Introduction

Radiation is one of the more dangerous clastogens in the environment. Ionizing radiation causes chromosome breakages and various cytogenetic aberrations in exposed cells [1]. In an investigation into radiation emergencies, it is important to estimate the dose to exposed persons for several reasons. Physical dosimeters (e.g., film badges) may misrepresent the actual radiation dose and may not be available in a radiological accident or terrorism incident. Biological dosimetry is suitable for estimating the radiation dose during such accidents [2]. The dicentric chromosome assay is very sensitive and a reliable bio-indicator in cases of accidental overexposure [3]. The purpose of the present experiment was to provide data on the dose-dependent production of chromosome aberrations such as dicentrics, centric rings, and excess acentrics. To establish the control dose response curve that is appropriate for our laboratory, external gamma radiation was provided by a ^{60}Co source (42.6 TBq of capacity; AECL, Canada at Korea Atomic Energy Research Institute).

2. Materials and Methods

2.1 Blood samples and radiation exposures

Human peripheral blood was collected from one healthy donor (female, 26 years old) without occupational exposure to harmful agents. A venipuncture heparinized whole blood sample of 10 ml was placed into a water bath and incubated at 37℃ for 1 hour immediately after irradiation *in vitro* by gamma rays (0-5 Gy, 5 min, room temperature).

2.2 Dicentric analysis

Dicentric analysis was performed by the cytogenetic procedure [4]. The whole blood cultures were prepared by adding 0.3 ml blood to 4 ml culture medium (RPMI-1640) to which antibiotic and Bromodeoxyuridine have already been added. 10% fetal bovine serum and 100 ㎕ phytohemagglutin (PHA-M) was added to mitosis and stimulate the lymphocyte proliferation. The blood samples were incubated in a cell culture flask (25 ㎠) at 37℃, 5% CO_2 in humidified atmosphere for 45 hours. Following incubation, a 50 ㎕ colcemid solution was added to the culture and shaken gently. This culture was maintained for 3 more hours to provoke mitotic arrest.

After lymphocyte culturing, cells were harvested by centrifugation (200 g, for 10 min) and treated with 0.075 M potassium chloride solution to lyse the red blood cells. The lymphocytes were fixed with Carnoy's solution, a mix of methanol : glacial acetic acid (3 : 1). The cells were washed twice with a fixative solution, and the cell suspension from each sample was then dropped onto a wet clean glass slide. The slides were stained with a fresh Giemsa solution and allowed to dry. Finally, the slides were mounted in DPX.

2.3 Conventional microscopy

Stained slides were scanned methodically at 200 fold magnification so that the entire area is covered. Once good quality metaphases were collected, dicentric analysis was conducted at high magnification (1,000 x). For a dicentric analysis, 500 complete metaphases with 46 centromeres were recorded. If the cell contains unstable aberrations, it should then balance. Tricentric aberrations are equivalent to two dicentrics and should have two accompanying fragments, while quadricentrics will have three fragments, and so on.

2.4 Statistics

A statistical analysis of the data was performed using Microsoft Office Excel 2010 and PASW Statistics 18. The calibration curve was made by fitting the data to a linear quadratic equation $Y = \alpha D^2 + \beta D + c$. Correlation coefficients (r) were calculated at a significant level of $P < 0.05$.

3. Results and Discussion

The unstable chromosome aberrations per 500 cells were analyzed, respectively, after scoring a total of 3,000 metaphases by a conventional method. Table 1 shows yields of dicentrics, centric rings and acentrics. The frequencies of dicentric and acentric chromosomes in the high-dose irradiation group were significantly higher than in the low-dose irradiation group. The centric ring was not found at a 0 Gy dose.

Table 1. Distribution of chromosome aberrations for different doses of γ-rays

Dose (Gy)	Number of dicentrics	Number of centric rings	Number of excess acentrics
0	1		1
1	251	23	156
2	322	20	304
3	405	26	439
4	493	17	513
5	834	81	915

A dose-related increase of the frequency of aberrations was observed. In particular, up to 4 dicentric chromosomes were found in a sample irradiated with 5 Gy gamma rays (Fig. 1). The dose-response curve of dicentric yields was expressed with a linear-quadratic equation, $Y = 0.0187D^2 + 0.1908D + 0.1204$ (r^2=0.93).

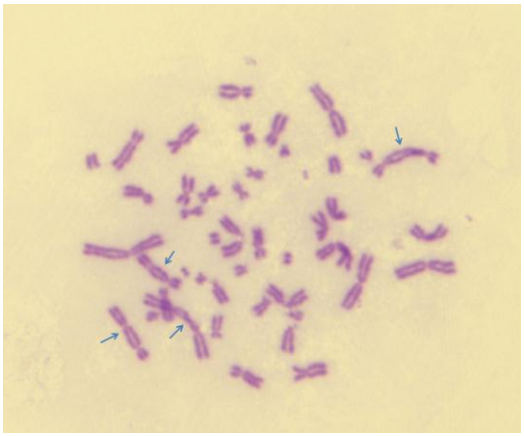

Fig. 1. Metaphase chromosome containing dicentrics (arrow) aberrations following 5 Gy irradiation.

This study demonstrated that the production of dicentrics in human lymphocytes was intimately related with the irradiation dose. We have already derived the dose response calibration curve from a cytokinesis-block micronucleus assay in our previous study [5]. Therefore, these cytogenetic analyses will be helpful to a dose estimation after radiation exposure. The population size increase is necessary to ensure the data reliability and accuracy in future studies.

REFERENCES

[1] S. A. Amundson, M. Bittner, P. Meltzer, J. Trent and A. J. Fornace, Jr, Biological indicators for the dentification of ionizing radiation exposure in bumans, Exper Rev. Mol. Diagn. 1(2), p.89-97, 2001.
[2] E. A. Ainsbury and J. F. Barquinero, Biodosimetric tools for a fast triage of people accidentally exposed to ionising radiation. Statistical and computational aspects. Ann. Ist. Super. Sanita. Vol.45, p.307-312, 2009.
[3] R. C. Wilkins, H. Romm, U. Oestreicher, L. Marro, M. A. Yoshida, Y. Suto, and P.G.S. Prasanna, Biolgical dosimetry by the triage dicentric chromosome assay - Futher validation of international networking, Radiation Measurements, Vol.46, p.923-928, 2011.
[4] International Atomic Energy Agency. Cytogenetic dosimetry: applications in preparedness for and response to radiation emergencies, Vienna, IAEA, 2011.
[5] T. H. Ryu, C. Roh and J. K. Kim, Cytokinesis-block micronucleus (CBMN) assay in human lymphocytes irradiated in vitro with gamma-rays, The Joint International Symposium on EPR Dosimetry and Dating and the International Conference on Biological Dosimetry, Mar,24-28, 2013, Leiden.