Dose Assessment using Chromosome Aberration Analyses in Human Peripheral Blood Lymphocytes

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1. Introduction

Ionizing radiation causes chromosome breakages and produces 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. Biological dosimetry using chromosome aberration analyses in human peripheral blood lymphocytes (HPBL) is suitable and useful tool for estimating the dose when a nuclear or radiological emergency is investigated [2~3]. The conventional analysis of dicentric chromosomes in HPBL was suggested by Bender and Gooch in 1962 [4]. This assay has been for many years, the golden standard and the most specific method for ionizing radiation damage [5]. The dicentric assay technique in HPBL has been shown as the most sensitive biological method and reliable bio-indicator of quantifying the radiation dose [6]. In contrast, the micronucleus assay has advantages over the dicentric assay since it is rapid and requires less specialized expertise, and accordingly it can be applied to monitor a big population [7]. The cytokinesis-block micronucleus (CBMN) assay is a suitable method for micronuceli measurement in cultured human as well as mammalian cells. The aim of our study was to establish the dose response curve of radiation-induced chromosome aberrations in HPBL by analyzing the frequency of dicentrics and micronuclei.

2. Materials and Methods

2.1 Radiation exposure and culture conditions

Human peripheral blood was collected from five healthy adult donors. A venipuncture heparinized whole blood samples were 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). The whole blood samples were cultured in RPMI-1640 containing 10% fetal bovine serum, antibiotic and phytohemagglutin (PHA-M) at 37℃, 5% CO_2 in humidified atmosphere.

2.2 Dicentric and CBMN analysis

Dicentric and CBMN analyses were carried out according to the cytogenetic procedure [8].

The dicentric analysis samples were incubated for 48 hours, including colcemid solution supplement for provoking mitotic arrest during the last 3 h.

The blood cultures of CBMN analysis were performed in cell culture flask for 24 hours. Following incubation, Cyt-B (Cytochrome B) was added to each sample. This culture was maintained for 48 hours to block cytokinesis and to induce binuclear cells.

After lymphocyte culturing, cells were harvested by centrifugation and treated with 0.075 M potassium chloride solution. The lymphocytes were fixed with Carnoy's solution. The cells were washed twice with fixative solution. A drop of cell suspension from each sample was spread onto a wet clean glass slide. Staining of the slides was done with fresh Giemsa solution. Finally, the slides were mounted in DPX after air drying. The stained slides were evaluated under a microscope at 200, 400 and 1,000 fold magnification.

2.3 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 $Y = \alpha D^2 + \beta D + c$.

2.4 Ethical considerations

The experiments including collection of blood samples from the voluntary donors were started before onset of the Bioethics and Safety Act of Korea (23 March 2013). However, all the procedures regarding this study were based on obtained consent and followed the legal and regulatory requirements for human experimentations.

3. Results and Discussion

The dicentric chromosomes and micronuclei of HPBL were analysed, after *in vitro* irradiated with gamma rays.

In a total of 21,688 analyzed metaphase spreads, 10,969 dicentric chromosomes, 563 centric rings and 11,364 acentric chromosomes were found. The number of dicentric, centric ring and acentric chromosomes in the high-dose irradiation group was higher than in the low-dose irradiation group. The centric rings were not

found at a 0 Gy dose, futhermore, there was not related with dose variation.

The frequency of total MN increased in a dose-dependent manner. In comparison with the control value, MN increased about 9, 32, 75, 87, and 52 fold higher after treatment with 1, 2, 3, 4, and 5 Gy, respectively.

The resulting dose-response calibration curves pooled from the five donors showed a classical linear-quadratic shape (Fig. 1). The curves were reported with 95% lower and upper confidence limits.

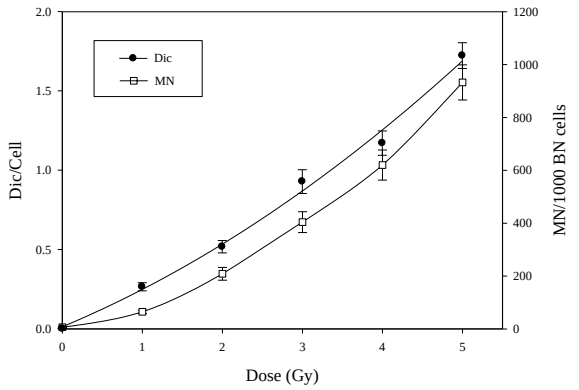

Fig. 1. The frequency of dicentrics (Dic) and micronucli (MN) induced by gamma rays in lymphocytes derived from five donors. Error bars represent the standard error.

The healthy five donors were recruited to establish the dose-response calibration curve for chromosomal aberrations by ionizing radiation exposure. Our cytogenetic results revealed that the mean frequency of chromosome aberration increased with increasing radiation dose. In this study, dicentric assay and CBMN assay were compared considering the sensitivity and accuracy of dose estimation. Therefore, these chromosome aberration analyses will be the foundation for biological dosimetric analysis with additional research methods such as translocation and PCC assay.

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