Comparison of Differences between Dicentric Assay and Translocation Analysis for Biodosimetry in Cultured Peripheral Blood Lymphocytes of Korean Individuals

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1. Introduction

Chromosome aberrations are considered to be important indicators of induced DNA damage and genomic instability. For this reason, they constitute the main parameter used to monitor individuals exposed to radiation. Biological dosimetry using the analysis of dicentrics in human lymphocytes is well established, especially in case of acute exposure, when the blood samples are taken within a few weeks (1). However, dicentric analysis is not an adequate parameter in case of chronic exposure, because these aberrations are unstable with time, and have a limited use for dose assessment of past exposures (2). In contrast to dicentrics, however, translocations are considered stable in cell division and so the yield should not fall with time. In the present study, using FISH-chromosome painting analysis with the dose-response curve for chromosome aberrations, we monitored the stable and unstable chromosome aberrations of 2 Korean's periperal blood lymphocytes irradiated in vitro with γ-rays from ^{137}Cs (doses between 0.0 and 2.0 Gy). By using the dose-response curve for chromosome aberration, our aim was to estimate the absorbed doses, and then establish comparison with the results obtained by conventional dicentric analysis, thus taking the opportunity to test the validity of chromosome aberration analysis by FISH painting method for retrospective biodosimetry in Korean individual.

2. Methods and Results

All procedures related to the collection and handling of blood samples from humans were performed with informed consent and according to the protocol approved by the Institutional Review Board.

2.1 Radiation exposure and Culture conditions

Peripheral blood samples from two healthy adult donors were collected in heparinized tubes (Becton-Dickinson). The blood samples were then exposed to γ-rays from a ^{137}Cs γ-ray source (Atomic Energy of Canada Ltd, Canada and located in Institute, Seoul, Korea) at a dose rate of 3.81 Gy/min. The exposed range was from 0.0 to 2.0 Gy. The blood samples were cultured in RPMI 1640 (Gibco) containing HEPES buffer, 15% heat inactivated fetal bovine serum, antibiotics, and phytohaemagglutinin (Gibco) at 37℃ in a humidified atmosphere containing 5% CO2.

2.2 Chromosomal preparations and FISH methods

The cultures were incubated for 48 h at 37℃, including colcemid treatment during the last 4 h. The chromosomal spreads were prepared by the conventional method. FISH analysis was carried out using different cocktails of specific cDNA probes; chromosome 4 and 18 were FITC labeled: chromosome 1 and 19 were biotinylated and detected with Texas-Red: Pan-centromere were labeled FITC or biotin. All probes were directly-labelled (Cambio) and used in accordance with the recommended protocol. DAPI-SlowFade mixture (Molecular Probes) was used for counter-staining. Chromosome aberrations were classified in accordance with classical nomenclature (PAINT: Protocol for Aberration Identification and Nomenclature Terminology) as shown in Tucker et. al. (3).

2.3 Statical analysis

The results obtained from the analysis of chromosome aberrations by conventional and FISH method were analyzed using the non-parametric variance test (Anova on Ranks), and the multiparametric comparison of Kruskal-Wallis. For the construction of dose-response curve, we used a weighted least squares method.

2.4 Conventional Dicentric assay and FISH analysis

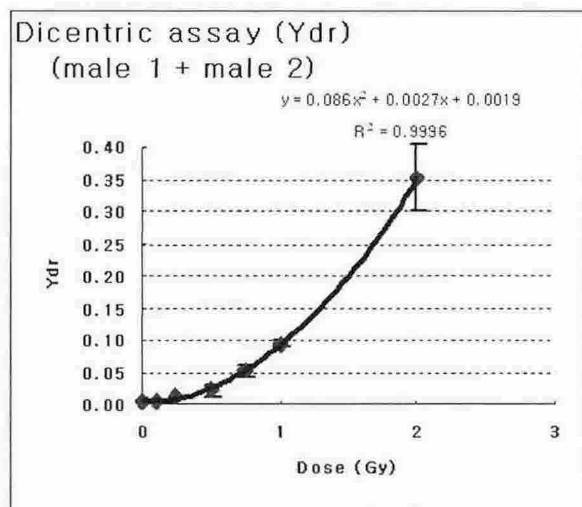

Figure 1. Ydr: $y=0.086x^2+0.0027x+0.0019$, $R^2=0.9996$

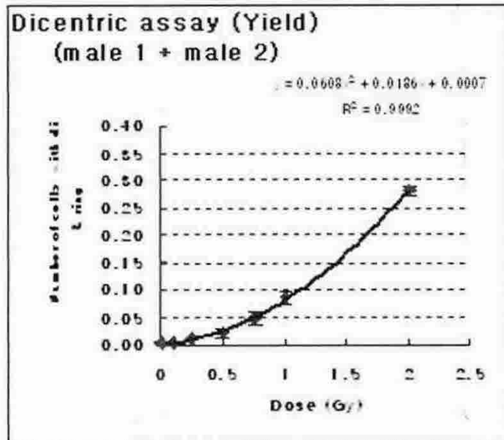

Figure 2. Yield: $y=0.0608x^2+0.0186x+0.0007$, $R^2=0.9992$

Dose assessment was performed by the frequencies of chromosome aberration measured by conventional dicentric assay to compare with the results of FISH-painting methods in two male Korean, respectively. In FISH-painting methods, dose assessment was performed by the frequencies of stable and unstable chromosome aberrations in chromosome number 1, 4, 18, and 19 in irradiated lymphocytes from two males, respectively. Stable chromosome aberrations contain translocations and insertions. Unstable chromosome aberrations contain dicentrics, rings, and acentric fragments. For the construction of a calibration curve of total chromosome aberrations, all types of aberrations (insertions, translocations, dicentrics, rings, and acentric fragments) were included.

Chromosome 1, 4, 18, 19 combination is 19.31% efficient in detecting translocations of the genome in male. Chromosomes #1 corresponded to 8.22%, chromosome #4 to 6.34%, chromosome 18 to 2.66%, and chromosome 2.09%, as estimated from the physical lengths of chromosomes (4). The dose-response curve for the total chromosome aberration frequencies by FISH methods in two male fits a linear quadratic models, according to the equation: $Y = 0.0238X^2 + 0.0098X + 0.0066$ ($R^2 = 0.9896$), for the stable chromosome aberration is $Y = 0.0106X^2 + 0.0055X + 0.0017$ ($R^2 = 0.9893$), and for the unstable chromosome aberration is $Y = 0.0132X^2 + 0.0043X + 0.0048$ ($R^2 = 0.9757$). The equation data of each male are below:

male 1: T: $Y = 0.0465X^2 - 0.0116X + 0.0116$ ($R^2 = 0.9827$)
U: $Y = 0.0245X^2 - 0.0053X + 0.0078$ ($R^2 = 0.9753$)
S: $Y = 0.0219X^2 - 0.0063X + 0.0038$ ($R^2 = 0.9841$)
male 2: T: $Y = 0.0011X^2 + 0.0312X + 0.0016$ ($R^2 = 0.9926$)
U: $Y = 0.0019X^2 + 0.0139X + 0.0019$ ($R^2 = 0.9482$)
S: $Y = -0.0008X^2 + 0.0172X - 0.0003$ ($R^2 = 0.9606$)

T means total translocations, U means unstable translocations, S means stable translocations.

FISH-painting methods in this work is feasible only for some exposed range (less than 2 Gy) in both total and stable chromosome aberration, even if it were so, it should be a need to additional sample numbers are needed to apply the biological dosimetry.

3. Conclusion

The in vitro dose–response curves for the genomic translocation frequencies and conventional dicentric assay fit a linear quadratic model, according to the equation each: $y = aX^2 + bX + c$. The values of FGs and dicentric assay were also calculated for the exposed range from 0.00 to 2.00 Gy for HPBL of the individuals exposed to ^{137}cesium on same samples, taking the opportunity to test the validity of translocation analysis in biodosimetry. A tentative of retrospective dosimetry was performed, indicating that the method is feasible only for some exposed range (below 2 Gy), while for higher doses there is a need to apply appropriate correction factors, which take into consideration mainly the persistence of chromosomal translocations along with time, and the influence of endogenous and exogenous factors determining the inter-individual variability in the cellular responses to radiation.

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