ESTABLISHMENT OF ^{60}CO DOSE CALIBRATION CURVE USING FLUORESCENT *IN-SITU* HYBRIDIZATION ASSAY TECHNIQUE: RESULT OF PRELIMINARY STUDY

Pembentukan Lengkuk Piawai Dos ^{60}Co
MenggunakanTeknik Asai Fluorescent in-situ Hibridisasi : Keputusan Kajian Awal

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Abstract

This study aims at establishing an in-vitro ^{60}Co dose calibration curve using Fluorescent In-Situ Hybridization assay technique for the Malaysian National Biodosimetry Laboratory. Blood samples collected from a female healthy donor were irradiated with several doses of ^{60}Co radiation. Following culturing of lymphocytes, microscopic slides are prepared, denatured and hybridized. The frequencies of translocation are estimated in the metaphases. A calibration curve was then generated using a regression technique. It shows a good fit to a linear-quadratic model. The results of this study might be useful in estimating absorbed dose for the individual exposed to ionizing radiation retrospectively. This information may be useful as a guide for medical treatment for the assessment of possible health consequences.

Keywords: *Fluorescent In-Situ Hybridization, in-vitro ^{60}Co dose calibration curve and translocation*

Abstrak

Kajian ini bertujuan untuk membina lengkuk piawai ^{60}Co in-vitro menggunakan teknik asai fluorescent in-situ hibridisasi untuk Makmal Biodosimetri Kebangsaan. Sampel darah diperolehi daripada seorang penderma wanita yang sihat dan diiradiasi dengan beberapa dos ^{60}Co. Setelah sel limfosit dikultur, kromosom disediakan atas slaid, proses didenaturasi dan hibridisasi dijalankan. Frekuensi translokasi kemudiannya dianggarkan di dalam setiap sel metafasa. Lengkuk piawai dihasilkan dengan menggunakan teknik regresi dan menunjukkan ia menuruti model linear-kuadratik. Keputusan kajian ini dijangka berguna dalam menganggarkan dos terserap untuk individu yang terkena sinaran mengion secara retrospektif. Maklumat ini juga dijangka berguna sebagai panduan rawatan perubatan untuk penilaian konsekuensi kesihatan yang mungkin berlaku.

Katakunci : *fluorescent in-situ hibridisasi, lengkuk piawai ^{60}Co in-vitro dan translokasi*

INTRODUCTION

Ionizing radiation can cause break to the single and double strand DNA, base damage and DNA cross-link proteins. These DNA damage will be repaired by the system of the body to return to normal chromosomes. However, the body may be failed or misrepaired to form normal or remain unrepaired. The double stranded breaks are the lesion responsible for most visible chromosomal aberration observed at metaphase after irradiation of normal cells. There are two type of chromosomal aberration namely unstable and stable aberration (IAEA, 2001).

Stable aberration, such as reciprocal (complete two-way) translocation is the exchange of terminal portion of two separate chromosomes (IAEA, 2001). This aberration will persist in the body many years and as an indicator for retrospective biological dosimetry. Solid giemsa staining cannot observe the translocation. The application of Fluorescence *in-situ* Hybridization (FISH) technique will visualize the translocation in the chromosome as bicoloured monocentric chromosome.

Scoring translocations by FISH with whole chromosome specific probes has been suggested to be an alternative method for biological dosimetry (B.S. Rao *et al.* 2001) FISH has been extensively used in attempts to assess past exposures to ionizing radiation in stable chromosomal aberration (C. Lindholm *et al.* 2004). FISH is a cytogenetic technique used to detect and localized the presence or absence of specific DNA sequence on chromosomes. FISH use fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence similarity. Fluorescent microscopy can be used to find out where the fluorescent probe bound to the chromosomes. In this method, the chromosomal rearrangement in translocation can be observed appropriately among the painted chromosomes. Practically, chromosome cocktail which usually consist of 3 pairs of larger chromosome ranging between chromosomes #1 to #12 can be selected for FISH painting in translocation observation and measurement.

Establishing the dose calibration curve is important for the reconstruction of radiation dose of expose radiation worker in standard biodosimetry laboratory. This study aims at establishing an in vitro ^{60}Co dose calibration curve using fluorescence in situ hybridization assay technique for the Malaysian National Biodosimetry Laboratory. The result of this study might be useful in estimating absorbed dose for the individual exposed to ionizing radiation retrospectively. This information may be useful as a guide for medical treatment for the assessment of possible health consequences.

MATERIALS AND METHODS

a) Irradiation

Ten ml of freshly taken blood specimens in lithium heparin tubes were collected from a female healthy donor. Blood was divided into 1ml aliqouts in lithium heparin tube for irradiated processed. Samples were irradiated at dose 0, 0.1, 0.15, 0.25, 0.5, 1.0, 2.0, 3.0, 3.5 and 4.0 Gy respectively using a ^{60}Co teleteraphy unit ELDARADO 8 # 104 located at the Secondary Standard Dosimetry Laboratory (SSDL). They were irradiated at a dose rate of 0.98 Gy min^{-1}. Four mm Polyvinyl chloride (PVC) is used as an absorbing material surrounding the lithium heparin tube to stimulate a body condition.

b) Slide preparation

After irradiation, blood samples were kept at 37°C for one hour to allow for any chromosomal repair take place. After that blood were cultured into 10 ml complete culture media, stimulated to divide by addition of 300 µl of 2.5 mg/ml stock phytohaemaglutinin (PHA) and incubated at 37°C with 5% carbon dioxide for 48 hours. At 45 hours incubation, 100 µl of 10 µg/ml stock colcemide was added to arrest cells in metaphase and will continuing incubation for another 3 hours. Following the full incubation period, the cultured blood cells was

harvested and fixed. Metaphases slides were prepare by dropping 2-3 drops of cell in fixative on grease-free slide and dry it for 24 hours. FISH assay procedure was carried out namely, denaturation of chromosome and probes, hybridization, washing and analysis. In this study, we choose to paint chromosome number 1, 3 and 9.

c) FISH analysis

Analysis was conducted on intact metaphases with good painting quality and with of all painted chromosome visible. Metaphases containing aberrations were captured and store in the computer. Frequencies of translocation were counted for cell with 46 chromosomes. Cell with the number of chromosome below than 46 were excluded. A total of 6 to 1000 cells were scored per dose point. Unfortunately more cells could not be scored in this preparation. All the translocations such as reciprocal translocations (complete), terminal translocation, insertion and inversion involving painted chromosome were scored. Each metaphase was examined under DAPI filter to distinguish between dicentrics and translocation.

d) Development of dose calibration curve

The *in-vitro* ^{60}CO dose calibration curve for the induction of translocation was generated based on the frequency of translocations involving chromosomes 1, 3 and 9 collectively. Frequency of dicentric was used for low dose part (<1.0 Gy) (IAEA, 2001), while frequency of FISH was used for high dose range ($\geq$ 1.0 Gy). Curve was then fitted to a linear quadratic model using regression technique.

RESULTS AND DISCUSSION

Figure 1 shows a photograph of FISH for chromosomes number 1, 3 and 9 and translocation occurred in chromosome number 1.

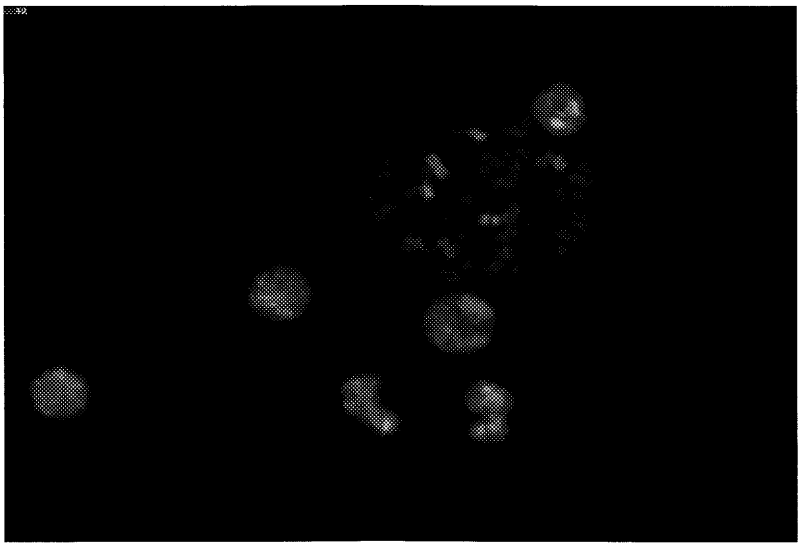

Figure 1. Painted chromosomes; Chromosome#1 painted in green, Chromosome #3 painted in red and Chromosome #9 painted in orange yellow. Also shows the translocation occurred at Chromosome #1.

The number of cell scored and frequency of translocation as in table 1.

Table 1. Number of translocation scored in chromosomes 1, 3 and 9.

Dose (Gy)	No. of cells scored	*Tc + Ti	Tc+Ti / Total cell score
0	1000	0	0
0.1	1000	0	0
0.15	1000	1	0.001
0.25	1000	3	0.003
0.5	1000	26	0.026
1.0	176	10	0.056
2.0	59	15	0.254
3.0	90	24	0.266
3.5	6	2	0.333
4.0	48	29	0.605

* Tc : complete translocation *Ti : incomplete translocation

For the construction of a calibration curve, all type of translocation was included. The dose calibration curve is fitted to linear quadratic model according to the equation: $Y = c + \alpha D + \beta D^2$, (E.Schmind *et al.*1992). Where, Y is the yield of aberration for dose D, C is the baseline aberration frequency in the control population, α and β are linear and quadratic coefficients respectively. Figure 2 shows the dose calibration curve for translocation in human lymphocyte exposed to radiation doses from ^{60}Co γ-ray. The yield of dose calibration is $Y= 0.0003 + 0.035X + 0.024X^2$ and fits to the linear quadratic model.

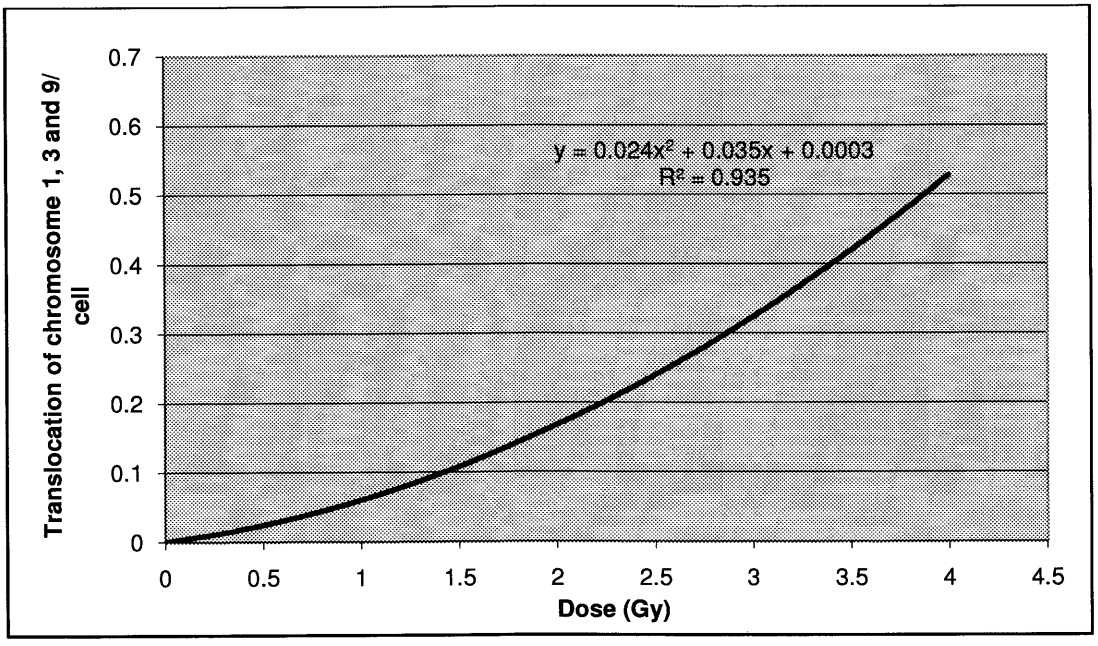

Figure 2. Dose calibration curve for translocation in human lymphocytes exposed to radiation doses from ^{60}Co γ-ray.

Table 2 shows result of comparison of the calibration curves parameters for translocation yield using ^{60}Co with result from other recent studies. It shows that α, which is linear coefficient and β, is quadratic coefficient are comparable with the value reported by E. Schmid *et al.* 1991 and C. Lindholm *et al.* 1998.

Table 2. Comparison of calibration curve parameters for translocation yield using ^{60}Co with the results from other recent study.

Source	C ± SE	α ± SE Gy^{-1}	β ± SE Gy^{-2}
E.Schmid *et al* 1991	0.00226 ± 0.00053	0.0094 ± 0.0047	0.0286 ± 0.0030
C.Lindholm *et al* 1998	0.0060 ± 0.0011	0.0061 ± 0.0092	0.00637 ± 0.0056
	0.0147 ± 0.0023	0.0113 ± 0.0134	0.0558 ± 0.006
Present study	0.0003	0.035	0.024

This study however is limited to only one donor. Future study should include samples from donors of different age because stable chromosome aberration is age dependent (IAEA, 2001).

CONCLUSIONS

We have established a dose calibration curve for the induction of translocations in human lymphocytes from blood irradiated with γ-ray in dose range of 0 – 4.0 Gy. This curve shows a good fit to a linear-quadratic model and might be useful in estimating absorbed dose for the individual exposed to ionizing radiation retrospectively.

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