ORIGINAL ARTICLE

Establishment of Dose-response Curves for Dicentrics and Premature Chromosome Condensation for Radiological Emergency Preparedness in Thailand

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ABSTRACT

The *in vitro* dose calibration curves using conventional biological dosimetry – dicentric chromosome assay (DCA) and premature chromosome condensation (PCC) assay – were performed for the first time in Thailand for reconstruction of radiation dose in the exposed individuals. The peripheral blood lymphocyte samples from healthy donors were irradiated with ^{137}Cs source at a dose rate of 0.652 Gy/min to doses of 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 Gy for DCA technique, and 5, 10, 15, 20, and 25 Gy for PCC technique. The blood samples were cultured and processed following the standard procedure as prescribed in the International Atomic Energy Agency report with slight modifications. The yield of dicentrics with dose from at least 1000 metaphases or 100 dicentrics was fitted to a linear quadratic model using Chromosome Aberration Calculation Software (CABAS, version 2.0) whereas those of PCC rings with dose from 100 rings was fitted to a linear quadratic equation at doses from 0 to 15 Gy. These curves will be useful for *in vitro* dose reconstruction and can support the preparedness for overexposure to radiation among public or occupational workers and eventual radiological accident in Thailand.

Key words: Cytogenetic biodosimetry, dicentrics, premature chromosome condensation, radiological emergency

Introduction

Due to increased utilization of radioactive materials and nuclear technologies in various fields, radiation accidents may be expected to occur at an unanticipated rate. In radiation emergencies, it is important to conduct a rapid dose estimation to apply the effective medical management. Biological dosimetry plays a valuable role by contributing in the early period after radiation emergencies. Thailand has experienced a serious radiological accident in Samut Prakan in 2000 when a disused ^{60}Co teletherapy head was partially dismantled, taken from an unsecured storage location and sold as scrap metal. That accident resulted in ten people receiving high radiation doses and three of those people died within 2 months of the accident as a consequence of their overexposure.[1] From the International Atomic Energy Agency's investigation of the accident, it was reported that there was apparently no adequate biological dosimetry to assess the probable range of the radiation doses received by the individuals involved.

Furthermore, at least five of the ten Southeast Asian countries, Indonesia, Malaysia, Philippines, Vietnam, and Thailand, are moving ahead with acquiring nuclear power plants. The time frame for the start of operation of the nuclear power plants extends from 2020. Therefore, radiation emergency preparedness including biological dosimetry concept has become a priority to establish support in the event of mass radiation casualties and to strengthen national and international radiation protection programs. The conventional cytogenetic assay using chromosome aberrations, such as dicentrics, fragments, and ring chromosomes, in an exposed cell can be regularly used for biological dosimetry. Dicentric chromosome assay (DCA) is an accepted method for whole-body individual dose assessment of <6 Gy whereas premature chromosome condensation (PCC) assay is appropriate in the high-dose range (>6 Gy).[2] The PCC assay can also discriminate between whole- and partial-body exposures at low doses.[3] The estimated

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dose can be derived from the constructed *in vitro* dose-response curves. Because of interlaboratory technical differences, laboratories performing biodosimetry should construct their own dose-response curves.[4] This study aimed to generate dose-response curves for dicentrics and PCC rings to cover low- and high-dose exposures to support radiation emergency preparedness in Thailand. The dose-estimation accuracy of those calibration curves was tested by performing an *in vitro* irradiation and blind scoring.

Materials and Methods

Blood collection and irradiation

The study was conducted in accordance with the guidelines prescribed by our Institutional Review Board. Volunteers were recruited from the faculty by informing the objectives and the experimental details of the study. The personal data including gender, age, alcohol and tea/coffee consumption, chronic disease, use of therapeutic drugs, and previous exposure to diagnostic X-ray were recorded in the questionnaire. Finally, three healthy nonsmoking volunteers were chosen as blood donors in the study. Written informed consent was obtained from each volunteer before the experiment. Blood samples from two donors were used for construction of dose-response curves and those from one donor were used for validation of curves.

Peripheral blood was irradiated with ^{137}Cs gamma rays (Gammacell 40 Exactor, MDS Nordion, Canada) at a constant dose-rate of 0.652 Gy/min with doses of 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 Gy for DCA, and 5, 10, 15, 20, and 25 Gy for PCC technique. The blood samples were further incubated for 2 h at 37°C to allow for repair of DNA damage.

Lymphocyte culturing and slide preparation

Whole blood cultures were set up using a standard protocol,[4] in RPMI 1640 medium supplemented with 20% fetal bovine serum and phytohemagglutinin and incubated at 37°C for 48 h. For DCA technique, colcemid was added to the culture at starting.[5] For PCC assay, calyculin A (50 nM) was added to the medium at the last 30 min before harvesting.[6] The cells were harvested with hypotonic treatment (0.075 M KCl) then fixed with Carnoy's fixative (methanol: glacial acetic acid, 3:1, v/v), dropped on a precleaned glass slide, air dried, and stained with 5% Giemsa solution in phosphate buffer.

Scoring

Chromosome aberrations were scored according to the criteria previously described in the literature.[4] A minimum of 100 dicentrics or 1000 cells were analyzed for DCA. A minimum of 80–100 PCC rings with a visible hole with or without centromere in G2/M-PCC cells were analyzed. Analysis of slides was carried out using an automated metaphase finding system (Carl Zeiss Axio Imager and Metafer supplied by MetaSystems, Germany).

Statistical analysis

The dose-response curves for DCA and PCC rings were fitted to linear quadratic equations using Chromosome Aberration Calculation Software (CABAS, version 2.0) (Institute of Nuclear Chemistry and Technology, Warsaw, Poland). The goodness of fit and homogeneity were determined using this software.

Results and Discussion

Dicentric chromosome analysis

Dicentrics were observed in metaphase chromosome in blood samples exposed to 0.1 to 5 Gy. For the analysis, only complete metaphases with 46 chromosomes were recorded. If the cell contained unstable aberrations, then it should be balanced. For example, a metaphase containing a dicentric should also have an acentric fragment, yet still count to 46 pieces. By contrast, a centric ring will also have an accompanying fragment, but the total number of objects in the cell will count to 47.[4] Metaphase chromosomes in control and exposed samples are shown in Figure 1. It is envisaged that tricentric aberrations are equivalent to two dicentrics and have two accompanying fragments, while tetracentrics will have three fragments.

The frequency and distribution of polycentric aberrations are shown in Table 1. The background level for dicentrics is very low as no dicentric was found in the 1000 cells analyzed. This implied that the larger number of scored metaphases is needed to see the dicentric yield and it accordingly requires a significantly longer period. From Table 1, it was shown that tricentrics were found at doses above 2.0 Gy whereas tetracentrics were found only in the dose of 5.0 Gy.

Table 1: Dicentric yield and distribution of polycentric chromosomes in ^{137}Cs gamma irradiated lymphocytes

Dose (Gy)	Number of cells scored	Polycentric chromosomes			Dicentrics/cell
		Dicentrics	Tricentrics	Tetracentrics	
0	1000	0	0	0	0
0.1	1001	6	0	0	0.006
0.25	1002	12	0	0	0.012
0.5	1000	23	0	0	0.023
0.75	1000	34	0	0	0.034
1	859	100	0	0	0.116
2	301	97	3	0	0.342
3	136	101	1	0	0.757
4	83	101	1	0	1.241
5	54	101	5	1	2.111
1.5*	647	100	0	0	0.155

*Curve testing dose

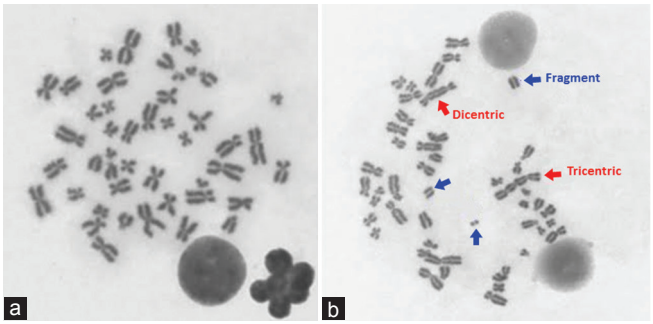

Figure 1: Metaphase chromosomes showing (a) normal chromosomes in control and (b) one dicentric, one tricentric, and three fragments in 3 Gy exposed samples

The dose-response curve was fitted by means of the CABAS, version 2.0, as illustrated in Figure 2. The dicentric yield has been shown to best fit to a linear-quadratic model as the following equation: $Y = aD^2 + bD + c$, where Y is the yield of dicentric frequency, D is absorbed dose in Gy, a is the corresponding quadratic coefficient = 0.0758 ± 0.0050, b is the linear coefficient = 0.0190 ± 0.0067, and c is the background frequency = 0 ± <0.0001.

This finding is in good agreement with the earlier reports.[7,8] Based on the resulting coefficients, the dose estimation was done by computing the number of aberrations observed and cells scored. When the given dose was 1.5 Gy, the dose calculated from CABAS was 1.31 Gy ranging from 1.17 to 1.46 Gy with 95% confidence limits.

Premature chromosome condensation analysis

The application of calyculin A-induced PCC rings has been widely used for dose estimation due to its advantage over conventional metaphase analysis for high-dose exposure that results in mitotic delay and disappearance of lymphocytes from peripheral blood circulation.[9] Calyculin A induces chromosome condensation in lymphocytes by specifically inhibiting protein phosphatases Type 1 and Type 2A (serine/threonine) in all phases of the cell cycle. The PCC morphology varies based on the stage of interphase in which the cells are present, namely, G1, S, and G2-PCC.[10] In our experiment, PCC rings were observed in the G2/M phase. To generate a calibration curve for PCC rings, a total of 500 cells or a minimal of 80–100 PCC rings per dose were scored.

Figure 3 shows an image of PCC rings in a sample exposed to 15 Gy. The G2/M-PCC rings were clearly observed because the elongated chromosomes enable easier visualization. The PCC ring frequency increased with the dose from 0 at 0 Gy to 1.15 at 15 Gy, and then slightly increased and saturated with the dose above 15 Gy as given in Table 2. This due to the mitotic delay was induced at the high doses from 15 to 25 Gy. These results are in good agreement with those reported in other studies.[9] The frequency of PCC rings with the doses from 0 to 15 Gy was fitted to a linear quadratic equation [Figure 4], where the corresponding quadratic coefficient: a = 0.0036 ± 0.0008, the linear coefficient: b = 0.0214 ± 0.0070, and the background frequency: c = 0 ± <0.0001. When the given dose was 7.5 Gy, the dose estimated from dose-response curve was 7.9 with its 95% confidence limit. The dose estimated ranged from 6.9 to 8.9 Gy.

Table 2: The yield of premature chromosome condensation rings induced in ^{137}Cs gamma irradiated lymphocytes

Dose (Gy)	Number of cells scored	Number of PCC-rings	PCC-rings/cell
0	500	0	0
5	482	96	0.199
10	193	106	0.549
15	100	115	1.150
20	89	105	1.180
25	72	85	1.181
7.5*	255	100	0.392

*Curve testing dose. PCC: Premature chromosome condensation

Conclusion

The dose-response curves of dicentrics and PCC rings for ^{137}Cs gamma radiation were established for the first time in our laboratory. These curves should be further developed and validated to be used for *in vitro* dose reconstruction in

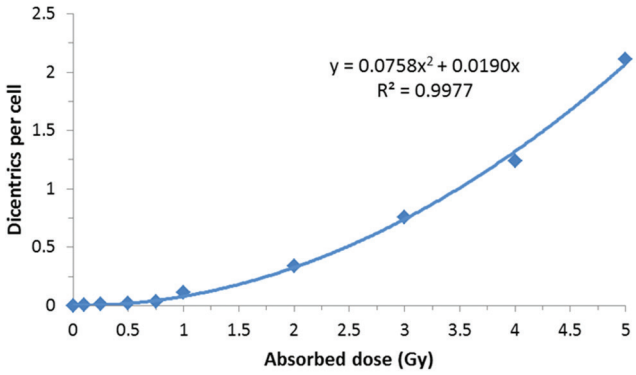

Figure 2: Dose-response curve for the induction of dicentrics in peripheral blood lymphocytes induced by ^{137}Cs gamma radiation

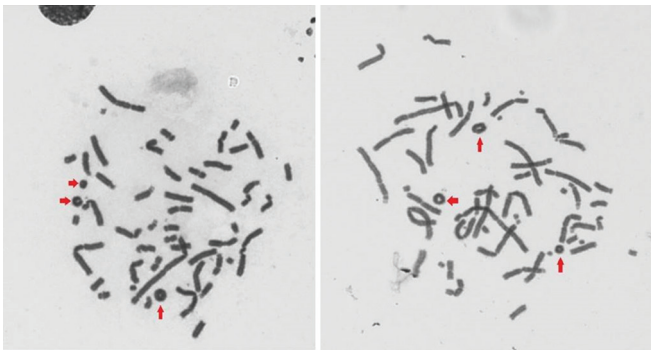

Figure 3: Two G2/M-premature chromosome condensation spreads with three premature chromosome condensation rings each in 15 Gy exposed sample

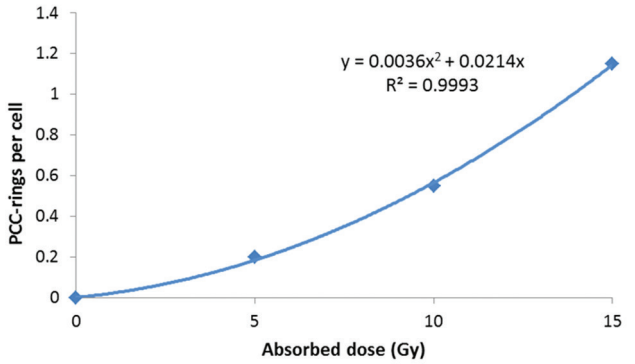

Figure 4: Dose-response curve for the induction of premature chromosome condensation rings in peripheral blood lymphocytes induced by ^{137}Cs gamma radiation

medical management in cases of radiological emergency in the country. The scoring of dicentrics and PCC rings is time consuming and highly expertise-dependent, and we lack experienced personal; therefore, the next step is to establish an in-house quality assurance program such as proficiency test of personnel qualifications, procedure manual, instrumentation, calibration, data reduction, record system, and data reporting as required to ensure the quality of a biological dosimetry laboratory's output.[4] Moreover, the International Organization for Standardization 19238 which provides standard criteria for service laboratories performing biological dosimetry by cytogenetics[11] should be adopted in the future.

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Conflicts of interest

There are no conflicts of interest.

References

1. IAEA. The Radiological Accident in Samut Prakan. Vienna: International Atomic Energy Agency; 2002.
2. Lindholm C, Stricklin D, Jaworska A, Koivistoinen A, Paile W, Arvidsson E, *et al.* Premature chromosome condensation (PCC) assay for dose assessment in mass casualty accidents. Radiat Res 2010;173:71-8.
3. Darroudi F, Natarajan AT, Bentvelzen PA, Heidt PJ, Van Rotterdam A, Zoetelief J, *et al.* Detection of total-and partial-body irradiation in a monkey model: A comparative study of chromosomal aberration, micronucleus and premature chromosome condensation assays. Int J Radiat Biol 1998;74:207-15.
4. IAEA. Cytogenetic dosimetry: Applications in preparedness for and response to radiation emergencies: EPR-biodosimetry. Vienna: International Atomic Energy Agency; 2011.
5. Hayata I, Tabuchi H, Furukawa A, Okabe N, Yamamoto M, Sato K. Robot system for preparing lymphocyte chromosome. J Radiat Res 1992;33:231-41.
6. Miura T, Blakely WF. Optimization of calyculin A-induced premature chromosome condensation assay for chromosome aberration studies. Cytometry A 2011;79:1016-22.
7. Griciene B, Slapšyte G, Mierauskiene J. Cytogenetic monitoring of nuclear workers occupationally exposed to ionising radiation. Radiat Prot Dosimetry 2014;159:10-9.
8. Pinto M, Amaral A. Biological dose assessment after low-dose overexposures in nuclear medicine. Radiat Prot Dosimetry 2014;162:254-9.
9. Balakrishnan S, Shirsath K, Bhat N, Anjaria K. Biodosimetry for high dose accidental exposures by drug induced premature chromosome condensation (PCC) assay. Mutat Res 2010;699:11-6.
10. Ravi M, Nivedita K, Pai GM. Chromatin condensation dynamics and implications of induced premature chromosome condensation. Biochimie 2013;95:124-33.
11. International Organization for Standardization. ISO 19238. Radiation Protection-Performance Criteria for Service Laboratories Performing Biological Dosimetry by Cytogenetics. Geneva, Switzerland: International Organization for Standardization; 2014.