Preparedness for Radiological Emergency in Thailand: Establishment of Dose-Response Curves for Dicentrics and PCC Rings

Benchawan Rungsimaphorn, Budsaba Rerkamnuaychoke, Wanwisa Sudprasert

ABSTRACT

The in-vitro dose calibration curves using conventional biological dosimetry: dicentric chromosome assay (DCA) and premature chromosome condensation (PCC) assay were pioneerly established in Thailand for reconstruction of radiation dose in the exposed individuals. The peripheral blood lymphocyte samples from healthy donors were gamma irradiated from $^{137}$Cs at a 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 technique, and 5, 10, 15, 20 and 25 Gy for PCC technique. The blood samples were cultured and processed following the standard procedure given by the IAEA with slight modifications. The yield of dicentrics with dose from at least 1,000 metaphases or 100 dicentrics was fitted to a linear quadratic model using Chromosome Aberration Calculation Software (CABAS, version 2.0), whereas those of PCC ring with dose from 100 rings was fitted to a quadratic equation at doses from 0-15 Gy. These curves will be useful for in-vitro dose reconstruction and can support the preparedness for public or occupational radiation overexposure and eventual radiological accident in the country.

Key words: cytogenetic biodosimetry, dicentric, premature chromosome condensation, radiological emergency.

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INTRODUCTION

Due to the increased utilization of radioactive materials and nuclear technologies applied for various fields, the radiation accidents are accordingly occurred by chance. In radiation emergencies it is important to estimate the dose rapidly in order that the effective medical management would be applied. Biological dosimetry plays a valuable role to contribute in the early period after a radiation emergency. Thailand has experienced a serious radiological accident occurred in SamutPrakarn 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 received high doses and three of those people died within two months of the accident as a consequence of their exposure (IAEA, 2002). From the IAEA’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 nuclear power plants. The time frame for the start of operation of the nuclear power plants is from 2020. Therefore, radiation emergency preparedness including biological dosimetry concept has become a priority to be setting up to support the event of mass radiation casualties and strengthen national and international radiation protection programs. The conventional cytogenetic assay using chromosome aberrations in an exposed cell such as fragments, dicentrics and ring chromosomes can be regularly used for biological dosimetry. Dicentric assay is the gold standard for whole body individual dose assessment of $< 6$Gy, whereas PCC ring assay is appropriate in the high-dose range ($>6$ Gy) (Lindholm et al., 2010). The PCC assay can also discriminate between whole- and partial-body exposures at low doses (Darroudi et al., 1998). The estimated dose can be derived from the constructed in vitro dose response curves. Because of inter-laboratory technical differences, laboratories performing biodosimetry should construct their own dose-response curves (IAEA, 2011). 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. The dose of 1.5 Gy and 7.5 Gy covering the possible low and high dose exposures were selected as blind testes for dicentric and PCC assay, respectively.

MATERIALS AND METHODS

1. Blood collection and irradiation

The study was conducted in accordance with standards of ethics. The written informed consent was obtained from each volunteer prior to the experiment. The personal data include gender,
age, smoking habits, alcohol and tea/coffee consumption, chronic disease, use of therapeutic drugs, previous exposure to diagnostic X-ray were recorded in the questionnaires.

Peripheral blood from healthy donor were irradiated with $^{137}$Cs gamma rays 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 technique, and 5, 10, 15, 20 and 25 Gy for PCC technique. The blood samples were further incubated for 2 h at $37^\circ$C to allow for repair of DNA damage.

2. Lymphocyte culturing and slide preparation

Whole blood cultures were set up using a standard protocol (IAEA, 2011), in RPMI 1640 medium supplemented with 20% FCS and PHA and incubated at $37^\circ$C for 48 h. For DCA assay, colcemid was added to the culture at starting (Hayata et al., 1992). For PCC assay, calyculin A (50nM) was added to the medium at the last 30 minutes before harvesting (Miura and Blakely, 2011). 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 pre-cleaned glass slide, air dried, and stained with 5% Giemsa solution in phosphate buffer.

3. Scoring

Chromosome aberrations were scored according to the criteria previously described (IAEA, 2011). A minimum of 100 dicentrics or 1,000 cells were analyzed for DCA. A minimum of 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).

4. Statistical analysis

The dose-response curve for DCA was fitted as a linear quadratic equation using Chromosome Aberration Calculation Software (CABAS, version 2.0). The goodness of fit and homogeneity were performed by this software. The PCC dose-response curve was fitted to a quadratic equation.

RESULTS AND DISCUSSION

1. Dicentric chromosome analysis

Dicentrics were observed in metaphase chromosome in blood samples exposed to 0.1 to 5 Gy. It was noted that only complete metaphases were recorded, i.e. those with 46 centromeres. 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 (IAEA, 2011). The pictures of metaphase chromosomes in control and exposed samples
were exhibited in Figure 1 as an example. It is appeared 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 were shown in Table 1. The background level for dicentrics is very low as no dicentric was found in 1,000 cells counted. This implied that the larger number of scored metaphases is needed to see the dicentric yield and it accordingly requires a significantly longer period of time. From Table 1, it was shown that tricentrics were found at doses above 2.0 Gy, whereas tetracentrics were found only the dose of 5.0 Gy.

The dose–response curve was fitted by means of the Chromosome Aberration Calculation Software (CABAS, version 2.0), as shown 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 = 0.075842 ± 0.00501, \( b \) is the linear coefficient = 0.019025 ± 0.00668, and \( c \) is the background frequency = 0 ± 0.00000.

This finding is in good agreement with the published papers by others (Griciene et al., 2014; Pinto and Amaral, 2013). Based on the resulting coefficients, the dose estimation was done by input 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 together with its 95% confidence limit. The dose estimated varied from 1.17 to 1.46 Gy.

![Figure 1 Metaphase chromosomes in control (0 Gy), 3 Gy and 5 Gy exposed samples.](image)
Table 1 Dicentric yield and distribution of polycentric chromosomes after $^{137}\text{Cs}$ gamma rays exposure.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>No. of cells scored</th>
<th>Polycentric chromosomes</th>
<th>Dicentrics/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dicentrics</td>
<td>Tricentrics</td>
</tr>
<tr>
<td>0</td>
<td>1000</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.1</td>
<td>1001</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>0.25</td>
<td>1002</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>1000</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>0.75</td>
<td>1000</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>859</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>301</td>
<td>97</td>
<td>3</td>
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<tr>
<td>3</td>
<td>136</td>
<td>101</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>83</td>
<td>101</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>101</td>
<td>5</td>
</tr>
<tr>
<td>1.5$^a$</td>
<td>647</td>
<td>100</td>
<td>10</td>
</tr>
</tbody>
</table>

$^a$ Curve testing dose

Figure 2 A dose-response curve for dicentrics generated by CABAS version 2.0 and the dose estimated from the software.
2. Premature chromosome condensation analysis

The application of calyculin A induced PCC rings have been widely used for dose estimation due to its advantage over conventional metaphase analysis when high dose exposure resulting in mitotic delay and disappearance of lymphocytes from peripheral blood circulation (Balakrishnan et al., 2010). 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 the G1, S and G2-PCC (Ravi et al., 2013). 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.

The example of pictures of PCC rings in 15 Gy exposed sample was exhibited in Figure 3. 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, 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 (Balakrishnan et al., 2010). The frequency of PCC rings with the doses from 0 to 15 Gy was fitted into a quadratic equation (Figure 4). When the given dose was 7.5 Gy, the dose estimated from dose-response curve was 7.19 Gy with its 95% confidence limit.

![Figure 3](image.png)

**Figure 3** G2/M-PCC cell in 15 Gy exposed sample showing 3 PCC-rings.
Table 2 PCC-ring frequency

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>No. of cells scored</th>
<th>No. of PCC-rings</th>
<th>PCC-rings/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>482</td>
<td>96</td>
<td>0.199</td>
</tr>
<tr>
<td>10</td>
<td>156</td>
<td>106</td>
<td>0.679</td>
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<td>15</td>
<td>100</td>
<td>115</td>
<td>1.150</td>
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<td>25</td>
<td>72</td>
<td>85</td>
<td>1.181</td>
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<tr>
<td>7.5(^a)</td>
<td>193</td>
<td>100</td>
<td>0.518</td>
</tr>
</tbody>
</table>

\(^a\) Curve testing dose

Figure 4 Dose response curve for the induction of PCC rings at 0-15 Gy.

CONCLUSION

The dose-response curves of dicentrics and PCC rings for \(^{137}\)Cs gamma radiation have been firstly established in our laboratory. These curves should be further developed and validated to be used for *in vitro* dose reconstruction in medical management in cases of radiological emergencies in the country. The scoring of dicentrics and PCC rings is time-consuming and highly expertise-dependent, whereas we are lacking of the experienced personal, therefore, in-house quality assurance program such as proficiency test of personnel qualifications, procedure manual, instrumentation, calibration, data reduction, record system and data reporting are required to ensure
the quality of a biological dosimetry laboratory’s output (IAEA, 2011). Moreover, International Organization for Standardization (ISO) 19238 which provides standard criteria for service laboratories performing biological dosimetry by cytogenetics (ISO 19238, 2004) should be adopted in the future.

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