Assessment of cell damages induced by gamma rays and pulsed electron beam at different dose rates

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The analysis of radiation induced micronuclei frequency in a binucleated cell (MN/BNC) is a standard biological end point to determine the cell damage as well as absorbed dose. In the present study, biological end points such as binucleated cells with micronuclei (BNC with MN) and the number of micronuclei per micro-nucleated binucleated cell (MN/(BNC with MN)) have been used to assess the cell damage. Further, the results of BNC with MN and MN/BNC with MN have been compared with those of previously obtained MN/BNC results. Cell damages have been assessed in human blood lymphocytes, exposed to ⁶⁰Co γ-rays at a dose rate of 0.033 Gy s⁻¹ and 8 MeV pulsed electron beam at a mean instantaneous dose rate of 2.6 × 10⁵ Gy s⁻¹. The relative biological effectiveness (RBE) for electrons in comparison with γ-rays, has been studied. Dosimeters such as fricke and ferrous ammonium benzoic acid and xylenol orange have been used for the dose measurements. The linear-quadratic dose response curves have been observed for both gamma and electrons. However, the relative effectiveness of 8 MeV electrons with that of gamma radiation for MN yield was found to be more than unity for all the biological endpoints studied at a given dose. Present study suggests that the quantification of BNC with MN and MN/(BNC with MN) along with MN/BNC provides additional information, while assessing radiation induced cell damage.

Keywords: Pulsed electron beam, γ-rays, Dose rate, Lymphocytes, Micronucleus

1 Introduction

The qualitative or quantitative estimations of the radiation induced damage in cells depend on particular biological endpoint studied. However, the reliability of the estimation also depend on the factors like radiation dose, energy, dose rate, LET (linear energy transfer) and cell line used for study. It is well known that chromosomal aberration analysis (CAA) and micronucleus (MN) assay are considered as standard methods to assess the radiation induced biological damages and well established as standard biological dosimeters¹,². Now, the aspects like fast scoring, comprehensiveness and reliability have made the MN assay as an easy alternate cytogenetic assay to CAA. From the studies, it is evident that the dose dependent MN yield is linear and liner-quadratic for high and low LET radiations, respectively³⁴⁵. However, the increase in likelihood for two or more chromosome fragments to coalesce into one MN, as dose increases and division delay of damaged cells at higher doses are few limitations of MN assay at higher doses⁶. The masking of MN by daughter nuclei is the other reason for the reduction of MN yield at high doses⁶. Therefore, while quantifying the MN induction within a cell, it seems important to take into an account of severity of the damages on cell population for better assessment of the cell damages.

In our earlier study, we obtained a liner-quadratic dose-response curve for MN/BNC, induced by 8 MeV pulsed electron beam at a mean instantaneous dose rate of 2.6 × 10⁵ Gy s⁻¹ and found higher effectiveness for electron as compared to γ-rays delivered at a dose rate⁵ of 0.033 Gy s⁻¹. In the preset study, attempt has been made to quantify BNC with MN and MN/(BNC with MN) along with MN/BNC provides additional information, while assessing radiation induced cell damage.

2 Materials and Methods

Microtron accelerator at Mangalore University, India, was used for electron irradiation. Dosimetry and irradiation of the blood samples have been carried out at 30 cm from the beam exit point of the accelerator. For study, blood samples were collected from a healthy 28 year old male donor by vein puncture in heparinized vial. Details of the accelerator
are given elsewhere. For gamma irradiation, Gamma Chamber-900 (BARC) was used. Dosimetry for the Gamma Chamber was carried out using FBX dosimeter.

In Microtron, the electron beam current and corresponding electron counts have been measured using fast current transformer and current integrator (CI), respectively. The calibration curve obtained for absorbed dose against CI counts was used to deliver the desired low doses in the present study. Details of the electron beam dosimetry for are given elsewhere.

MN assay as described by Fenech et al. with a few modifications was used in the present study. Details of the MN assay and preparations of MN slides and scoring criteria are given elsewhere.

3 Results and Discussion

A detailed investigation on response of the lymphocytes to 8 MeV pulsed electrons at a high dose rate was carried out by quantifying the MN frequency. The results of BNC with MN and MN/(BNC with MN) are presented in Table 1.

The dose-response curves for the induction of MN/(BNC with MN) and BNC with MN in lymphocytes induced by 8 MeV electrons and γ-rays are shown in Figs 1 and 2, respectively. In Fig. 3, dose-response curve obtained earlier for MN/BNC is also shown for comparison.

The data shown (Table 1) were fitted, using an error-weighted minimum chi² (χ²) method, to the linear-quadratic model represented by:

\[ y = C + \alpha D + \beta D^2 \]

where \( y \) is the corresponding measure of damage for the absorbed dose of \( D \) Gy, \( C \) represents the level of spontaneous damage at zero dose and \( \alpha \) and \( \beta \) are linear and quadratic coefficients, respectively. In each case, the background \( C \) values observed at zero doses for the corresponding measure of damage were fixed.
while fitting the curve. It is evident from Figs 1 and 2 that the fraction of BNC expressing MN and MN/(BNC with MN) followed the same pattern of response obtained earlier for MN/BNC (Fig. 3). The values of the parameters $\alpha$ and $\beta$ of the fitted curves, for all the three end points, are presented in Table 2.

In our earlier study, it was found that the values of MN/BNC induced by an individual electron dose were higher when compared with those of $\gamma$-rays. However, the $\beta$ coefficients obtained for electrons and $\gamma$-rays from the dose response curves were not significantly different within the experimental conditions used\(^5\). Similarly, the present study also shows a non-significant difference in the $\beta$ coefficients of the dose-response curves obtained for electrons and $\gamma$-rays, when the 95% confidence interval was considered for the endpoint BNC with MN (Fig. 1). Supporting these findings, the $\beta$ coefficients of the curves for the endpoint MN/(BNC with MN) and a non-significant difference are shown in Fig. 2. BNC with MN is an indication of average damage inflicted to a cell population, since it consists of scoring the fraction of BNC containing MN regardless of the number of MN in these BNC. On the other hand, MN/BNC is an expression of individual damage sustained in the affected cells.

The variations in the RBE with electron doses and RBE ($\alpha_{e}/\alpha_{\gamma}$) values are presented in Table 3. The RBE is dependent on electron dose and varied from 1.128 to 1.139 for MN/BNC, 1.294 to 1.114 for BNC with MN and 1.213 to 2.402 for MN/(BNC with MN), for the doses 1 to 6 Gy. In addition, the value of RBE ($\alpha_{e}/\alpha_{\gamma}$) determined as the ratio of $\alpha$ coefficients of the dose-response curves of electrons and $\gamma$-rays was found to be 1.12 ± 0.270 for MN/BNC, 1.35 ± 0.229 for BNC with MN and 0.77 ± 0.678 for MN/(BNC with MN), respectively.

From Table 3, it can be seen that for all the three endpoints, the RBE was found to be higher than unity, indicating electrons are more effective in inducing cell damage per unit absorbed dose as compared to that of $\gamma$-rays.

In the case of BNC with MN as an endpoint, the RBE values were found to decrease with electron dose (Table 3). It indicates that even though the average damage induced to a cell population (BNC with MN) by electrons and $\gamma$-rays increases with dose, the rate of increasing of BNC with MN induction is higher for $\gamma$-rays. However, the rate of damage per cell (MN/BNC) induced by $\gamma$-rays is less as compared to electrons for a given dose. The variations of RBE with electron dose determined from three endpoints (MN/BNC, BNC with MN and MN/(BNC with MN)) in irradiated blood lymphocytes are shown in Fig. 4.

### Table 2 — Values of the coefficients of linear-quadratic model fit for the fraction of BNC with MN, MN/BNC and MN/(BNC with MN)

<table>
<thead>
<tr>
<th>Radiation quality</th>
<th>$\alpha$ (Gy$^{-1}$)</th>
<th>$\beta$ (Gy$^{-2}$)</th>
<th>C</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN/BNC [previous study (Ref.5)]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\gamma$-rays</td>
<td>0.075 ±0.011</td>
<td>0.016 ±0.003</td>
<td>0.084 ±0.021</td>
<td>0.025</td>
</tr>
<tr>
<td>Electrons</td>
<td>±0.016</td>
<td>±0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNC with MN</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\gamma$-rays</td>
<td>8.496 ±1.036</td>
<td>0.394 ±0.207</td>
<td>11.506 ±0.148</td>
<td>2.35</td>
</tr>
<tr>
<td>Electrons</td>
<td>1.350 ±0.247</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MN/(BNC with MN)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\gamma$-rays</td>
<td>0.026 ±0.012</td>
<td>0.001 ±0.002</td>
<td>1.063 ±0.013</td>
<td>1.063</td>
</tr>
<tr>
<td>Electrons</td>
<td>0.020 ±0.013</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* $R^2$ represents the coefficients of determination of the curve

### Table 3 — RBE values of 8 MeV electrons as compared to $^{60}$Co $\gamma$-rays along with RBE ($\alpha_{e}/\alpha_{\gamma}$) values

<table>
<thead>
<tr>
<th>Electron dose (Gy)</th>
<th>RBE for various endpoints</th>
<th>$\alpha_{e}/\alpha_{\gamma}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN/BNC</td>
<td>BNC with MN</td>
<td>MN/(BNC with MN)</td>
</tr>
<tr>
<td>1</td>
<td>1.128</td>
<td>1.294</td>
</tr>
<tr>
<td>2</td>
<td>1.132</td>
<td>1.245</td>
</tr>
<tr>
<td>3</td>
<td>1.135</td>
<td>1.205</td>
</tr>
<tr>
<td>4</td>
<td>1.137</td>
<td>1.170</td>
</tr>
<tr>
<td>5</td>
<td>1.138</td>
<td>1.140</td>
</tr>
<tr>
<td>6</td>
<td>1.139</td>
<td>1.114</td>
</tr>
</tbody>
</table>

Fig. 4 — Variation in RBE as a function of dose of 8 MeV electrons for three end points. RBE values calculated relative to data of $^{60}$Co $\gamma$-rays
Furthermore, the increased RBE with dose in the case of MN/(BNC with MN) also indicates higher rate of damage to a cell population with lesser MN yield per individual cell with dose of gamma. On the other hand, for electrons, the MN yield increases with increasing rate of damage per cell rather than the increasing damage to a cell population.

The higher RBE \((\alpha_e/\alpha_\gamma)\) for BNC with MN and lower RBE \((\alpha_e/\alpha_\gamma)\) for MN/(BNC with MN) suggest that the effectiveness of 8 MeV electrons in the lower dose region results from the increase of damaged cell populations. Similar report can be seen for the induction of MN in human mammary epithelial cell line MCF-12A exposed to 25 kV X-rays\(^{10}\).

4 Conclusions

The quantification of BNC with MN and MN/(BNC with MN) is a continuation study of the earlier study done by scoring MN/BNC. Since MN yield decreases as severity of the damage within the cell increases, it seems important to quantify the MN induction in the cell population for reliable assessment. However, the estimation of number of mononucleated cells and the ratio of trinucleated to tetranucleated cells along with present results would provide precise assessment of the cell damage.

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