The relative biological effectiveness of 100 kV X-rays determined by the V-79 cell colony assay

E. WINZEL, E. J. VAN DER MERWE, W. GROENEWALD, S. PISTORIUS, J. P. SLABBERT, L. ROBINSON, L. BÖHM

Summary

The relative biological effectiveness (RBE) of 100 kV X-rays compared with cobalt-60-γ-irradiation was determined using a cell colony assay based on the survival of Chinese hamster V-79 lung fibroblasts. The 57% dose (D57) was found to range from 1.12 to 1.47 and from 1.33 to 1.63 Gy for X-rays and 60Co-γ-irradiation respectively. The mean RBE value calculated from the D57 values was found to be 1.13 ± 0.04. This figure compares favourably with RBE values calculated from D10 values using other endpoints.

Materials and methods

Chinese hamster lung fibroblasts V79 - 379A were grown in monolayer culture at 37°C, 5% CO2 in minimum essential medium (MEM) supplemented with 10% v/v fetal bovine serum, penicillin 100 U/ml, streptomycin 100 μg/ml and tylosin at 0.5%. Semi-confluent cells were harvested with trypsin 0.05% for 2 minutes and washed with medium containing 10% fetal bovine serum and counted. Cells were irradiated in 15 ml tissue culture tubes at a concentration of 1 x 10⁶ in 1 ml of MEM medium. For the 10 Gy dose the concentration of cells was 2 x 10⁵ and 3 x 10⁵ cells/ml respectively. Cells were kept on ice and irradiated at 0°C with 100 kV X-rays (2 mm Al-filter, half-value layer = 3.2 mm). The sample to filter distance was approximately 2 cm giving a dose rate of 5.09 Gy/min measured with an ionisation chamber. Cells were transported on ice, diluted and plated in triplicate in T-25 Falcon flasks containing 10 ml of medium to give approximately 200 colonies. After 5 days of incubation cells were fixed, stained with 0.01% Amidoblack in 20% acetic acid, 20% methanol and 60% water and colonies were counted. Survival was plotted against dose over two decades.

For the calculation of D57, data were fitted to the expression

\[ \text{S (D)} = e^{-\alpha D + \beta D^2} \]

where S = surviving fraction; D = dose; \( \alpha \) = single event inactivation coefficient; and \( \beta \) double event inactivation coefficient as defined.8

The mean inactivation dose \( \bar{D} \) defined by Fertil8 is given by the survival curve

\[ \bar{D} = \int_{0}^{\infty} \text{S (D)} \, dD \]

\( D_{57} \) is defined as the 37% dose or the dose required to reduce the surviving fraction by a factor of 1/e. \( D_{57} \) was derived from the single-hit multitarget equation:

\[ S = 1 - \left( 1 - \frac{D_{57}}{D} \right)^n \]

where \( S \) = surviving fraction; \( D \) = dose; \( D_{57} \) = 37% dose; and \( N \) = extrapolation number as defined.8

An AECL Theratron was used for 60Co-γ-irradiation at a dose rate of 3.67 Gy/min and a field size of 20 x 30 cm.

The individual \( D_{57} \) measurements as given in Table I were used to calculate the RBE according to the expression:

\[ \text{RBE} = \frac{D_{57} (60\text{Co-}\gamma\text{-irradiation})}{D_{57} (100 \text{ kV X-rays})} \]

and is given with 1 SE.

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have determined RBE-values for available 100 kV X-rays using 60Co-γ-irradiation as reference. The results have been analysed in respect of two current models of cell inactivation: the \( \alpha, \beta \) model developed by Chadwick and Leenhouts8 and Kellнер and Rossi2 and the single-hit multitarget model of Tumoffeeff-Resovsky and Zimmer.6 It was found that 100 kV X-rays are biologically 1.13 ± 0.04 times more effective than 60Co-γ-irradiation. This is consistent with the linear energy transfer (LET) values of the compared irradiation types and in good agreement with published RBE-fatures.
The statistical treatment was as follows: the errors for cell plating and for the colony counts were combined to derive the error for the plating efficiency. The plating efficiency error at zero dose (control) and at each dose point were then combined to give an estimate of the error associated with the surviving fraction and this is represented in the form of the error bars shown in Fig. 1A. A line was fitted to the points by computer with the instrumental weighting depending on the uncertainty associated with each data point. The uncertainty in the slope was then used to derive the error associated with each D0 measurement. For calculating the RBE, fractional errors in D0 were added in quadrature and multiplied by the RBE to yield the absolute error of the RBE at 68% confidence level. The weighted mean and uncertainty of the RBE were then calculated where each RBE value was weighted inversely by its variance according to a procedure described by Bevington.19

**Results and discussion**

A set of survival data from a paired experiment plotted according to the single-hit multitarget model is shown in Fig. 1A. It is evident that dose per dose fewer cells survive after irradiation with 100 kV X-rays than with 60Co-γ-irradiation. This is also shown by the D0 values which have been determined from a total of 5 paired experiments (Table I). From the individual RBE measurements we calculate an average RBE of 1,13 and an absolute error of 0,04. This, and the fact that the individual RBE values were all significantly above 1, demonstrates that the 100 kV X-rays are indeed the biologically more effective irradiation.

The data from 3 sets of paired experiments were also analysed according to the αβ model. The remaining 2 experiments could not be included here because of insufficient data points at low dose. The average RBE calculated from the corresponding D0-values were found to be 1,15 (Table II). A graph of 1 data set is shown in Fig. 1B. In view of the limited data available no statistical analysis was undertaken for these measurements. Our conclusions concerning the RBE of 100 kV X-rays are thus solely based on the calculations using the single hit multitarget model. We have previously analysed the relative merits of the αβ model — and the single hit multitarget model showing that the D0 parameter is more variable and hence less suitable.11,12

The RBE value of 1,13 obtained here for V79 cell survival compares favourably with the data of Sinclair and Blackwell13,14 using other endpoints. The RBE of 200 kV X-rays v. 60Co-γ-irradiation derived from the median lethal dose LD50(30) of rats was found to be 1,09 - 1,18,13,17 was derived from iron-59 uptake15 and 1,08 was obtained from the LD50 of 4-day-old chicken embryos.15 Hering16 in comparing 100 kV X-rays with 60Co-γ-irradiation found RBEs of 1,22 and 1,23 for mouse skin reactions after irradiation at 0 and 2,5 cm depth respectively. The trend of the RBE of kilovoltage X-rays to exceed 1 reflects the higher LET.17

Cell survival curves have long emerged as the yardstick by which radiation modalities are compared. We report that this assay is well capable of measuring small differences in RBE at the level of 1. We therefore expect the V-79 colony assay to be well capable of measuring the much greater RBE values expected for the high energy neutron beam at Faure.

**Table I. RBE of 100 KV X-Rays v. 60Co-γ-Irradiation Determined from V-79 Cell Survival Data Expressed According to the Multitarget Model**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>D0 (X-rays)</th>
<th>D0 (60Co-γ)</th>
<th>RBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1,33 ± 0,05</td>
<td>1,21 ± 0,06</td>
<td>1,10 ± 0,07</td>
</tr>
<tr>
<td>2</td>
<td>1,63 ± 0,10</td>
<td>1,47 ± 0,06</td>
<td>1,11 ± 0,08</td>
</tr>
<tr>
<td>3</td>
<td>1,38 ± 0,17</td>
<td>1,12 ± 0,08</td>
<td>1,23 ± 0,18</td>
</tr>
<tr>
<td>4</td>
<td>1,33 ± 0,08</td>
<td>1,22 ± 0,07</td>
<td>1,09 ± 0,09</td>
</tr>
<tr>
<td>5</td>
<td>1,37 ± 0,06</td>
<td>1,16 ± 0,06</td>
<td>1,18 ± 0,08</td>
</tr>
<tr>
<td>Mean</td>
<td>1,13 ± 0,04*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Weighted mean and weighted uncertainty (see 'Materials and methods').

**Table II. RBE of 100 KV X-Rays v. 60Co-γ-Irradiation Determined from V-79 Cell Survival Data Expressed According to the αβ-Model**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>X-rays</th>
<th>60Co-γ-irradiation</th>
<th>RBE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0,256 0,024 2,76</td>
<td>0,181 0,033 3,01</td>
<td>1,09</td>
</tr>
<tr>
<td>3</td>
<td>0,239 0,032 2,66</td>
<td>0,185 0,022 3,35</td>
<td>1,26</td>
</tr>
<tr>
<td>4</td>
<td>0,234 0,027 2,82</td>
<td>0,150 0,037 3,11</td>
<td>1,10</td>
</tr>
<tr>
<td>Mean</td>
<td>0,234 0,027 2,82</td>
<td>0,150 0,037 3,11</td>
<td>1,10</td>
</tr>
</tbody>
</table>

Fig. 1. Survival of Chinese hamster V-79 lung fibroblasts after exposure to various doses of photon irradiation. A. Data were plotted according to the single-hit multitarget model: – O – = 100 kV X-rays (y = 8,95 × 10^{-3} x + 3,26; D0 = 1,12 ± 0,08 Gy); O–O–O– = 60Co-γ-rays (y = 7,24 × 10^{-3} x + 2,54; D0 = 1,38 ± 0,17 Gy). B. Data were plotted according to the linear quadratic model: – O – = 100 kV X-rays (y = e^{-0,234 D} + 0,027 D^2; D0 = 2,82); O–O–O– = 60Co-γ-rays (y = e^{-1,050 D} + 0,037 D^2; D0 = 3,11)).
Non-invasive assessment of lower limb ischaemia by blood velocity wave-form analysis


Summary
Clinical examination combined with angiography is conventionally used to assess lower limb arterial disease. The shape of the blood velocity wave form in the common femoral artery varies with the extent of proximal arterial disease, suggesting that wave-form analysis may provide additional haemodynamic information of potential value in surgical decision-making. This paper studies the use of two methods of wave-form analysis, pulsatility index and Laplace transform analysis, to assess lower limb arterial disease. The blood velocity wave form was measured non-invasively at the common femoral artery using a locally developed mean frequency processor and a commercial 9.5 MHz bidirectional Doppler ultrasound unit. Wave forms from 70 limbs (35 patients) with suspected atherosclerotic arterial disease and from 20 normal limbs with no history or signs of disease were studied. Both methods of wave-form analysis provided a statistically significant separation between patients with severe and moderate disease as assessed angiographically (P < 0.001).

These results suggest that significant aorto-iliac disease can be virtually excluded by a normal common femoral wave form. Furthermore, wave-form analysis may have an important role in the follow-up of patients after bypass grafting or iliac angioplasty and in the detection of presymptomatic aorto-iliac disease.

In patients presenting with incapacitating claudication or rest pain, in whom femoral pulses are weak or absent and significant aorto-iliac disease is confirmed angiographically, the need for a proximal bypass procedure is usually obvious, and there is no diagnostic problem. However, a frequent problem is the case...