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# Characterization of relative biological effectiveness for conventional radiation therapy: a comparison of clinical 6 MV X-rays and $^{137}$Cs

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## ABSTRACT

Various types of radiation are utilized in the treatment of cancer. Equal physical doses of different radiation types do not always result in the same amount of biological damage. In order to account for these differences, a scaling factor known as the relative biological effectiveness (RBE) can be used. $^{137}$Cesium ($^{137}$Cs) has been used as a source of radiation in a significant body of radiation therapy research. However, high-energy X-rays, such as 6 MV X-rays, are currently used clinically to treat patients. To date, there is a gap in the literature regarding the RBE comparison of these two types of radiation. Therefore, the purpose of this study was to investigate the RBE of $^{137}$Cs relative to that of 6 MV X-rays. To determine the RBE, five cell lines were irradiated [Chinese hamster ovary (CHO); human lung adenocarcinoma (A549); human glioma (U251); human glioma (T98); and human osteosarcoma (U2OS)] by both types of radiation and assessed for cell survival using a clonogenic assay. Three of the five cell lines resulted in RBE values of ~1.00 to within 11% for all survival fractions, showing the physical and biological dose for these two types of radiation were equivalent. The other two cell lines gave RBE values differing from 1.00 by up to 36%. In conclusion, the results show the range in biological effect seen between cell lines, and therefore cell type must be considered when characterizing RBE.

**KEYWORDS:** relative biological effectiveness, radiotherapy, 6 MV X-rays, $^{137}$Cesium

## INTRODUCTION

Radiation therapy is a common modality used in the treatment of cancer. Various types of radiation are used for treatment, based on how different radiation types interact. For an equal physical dose, not all radiation types cause the same amount of biological damage. In order to account for the differences in biological damage, a scaling factor known as the relative biological effect (RBE) is utilized. Specifically, the RBE is a ratio of physical doses that generate the same damage and can be calculated by:

$$\mathrm{RBE} = D_{\mathrm{control}}/D_{\mathrm{test}} \quad (1)$$

In this equation $D_{\mathrm{control}}$ is the physical dose of a known radiation modality (i.e. X-rays) and $D_{\mathrm{test}}$ is the physical dose of the radiation modality being investigated [1].

In biological research, $^{137}$Cesium ($^{137}$Cs) is a commonly used source of radiation, as $^{137}$Cs irradiators are compact, affordable and readily available for research. $^{137}$Cs has been used in numerous studies to further our knowledge of the cellular and molecular responses and changes that occur due to radiation [2–11]. However, $^{137}$Cs is not commonly used in the treatment of patients; rather, high-energy (6 MV) X-rays are used. The results of this study may allow the knowledge gained using $^{137}$Cs to be translated to clinical practice using 6 MV X-rays.

The RBE of therapeutic X-rays (6 MV) and γ-rays [$^{137}$Cs, $^{60}$Cobalt ($^{60}$Co)] has been taken as 1.00, as both are sparsely ionizing types of radiation. However, previous research has shown this is not always the case. A study involving mouse jejunal crypt cells reported RBE values with $^{60}$Co as the reference radiation for $^{137}$Cs as 1.07 (0.92–1.26) and 4 MV X-rays as 1.13 (0.99–1.30) [12].

Therefore, the assumption that all X-rays cause the same biological effect may not be entirely accurate, and further study is needed.

Slight differences in certain physical characteristics [such as linear energy transfer (LET), mean energy and dose rate] of various X-ray radiation types may contribute to the varied RBE values previously reported. $^{137}$Cs-emitted photons and 6 MV X-rays have LET values, mean energies and dose rates that are not identical. The International Commission of Radiation Protection Report 92 states the importance of considering the LET of each type of radiation when evaluating the RBE, as the LET of X-rays and γ-rays can vary significantly. For example, the LET of $^{137}$Cs γ-rays is ~0.8 keV/μm compared with that of 200 kV X-rays (~3.5 keV/μm) [1]. Previously published values for clinical beams suggest a LET value of ~0.2 keV/μm for 6 MV X-rays [13]. Additionally, the mean energies of $^{137}$Cs-emitted photons and 6 MV X-rays at 10 cm depth are 662 keV and 2 MeV, respectively. Finally, the dose rates of these two radiation types were intentionally chosen so as to avoid a dose rate dependency, but are slightly different, as $^{137}$Cs had a dose rate of 413 cGy/min and 6 MV X-rays had one of 360 cGy/min at 10 cm depth. Though subtle, these differences in LET, mean energy and dose rate could lead to an RBE value that is not 1.00.

The purpose of this investigation was to measure the RBE of $^{137}$Cs with respect to 6 MV X-rays, as a direct comparison between $^{137}$Cs and 6 MV X-rays has not been performed in human cancer cell lines. We studied the biological response of five cell lines to radiation from both $^{137}$Cs and 6 MV X-rays in order to clarify and verify the measured RBE value for these two types of radiation, thus improving understanding of the translation of findings from lab data from previous $^{137}$Cs radiation studies to clinical practice with megavolt X-rays.

## MATERIALS AND METHODS

### Cell lines and culture conditions

Five types of cells were used in this study: Chinese hamster ovary (CHO); human lung adenocarcinoma (A549); human glioma (U251); human glioma (T98); and human osteosarcoma (U2OS). CHO and A549 cells were grown in F12-K medium supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin. U251 and T98 cells were grown in DMEM essential medium with 10% fetal bovine serum and 1% penicillin–streptomycin. U2OS cells were grown in McCoy's medium with 10% fetal bovine serum and 1% penicillin–streptomycin. Cell lines were purchased and identities verified from the ATCC (Manassas, VA, USA). All cell types were routinely subcultured every 2–5 days in conventional 75-cm$^2$ flasks (Corning, NY, USA) to ensure exponential growth, and kept in humidified conditions at 37°C and 5% $CO_2$. Cells were then counted and seeded in triplicate wells.

### Clonogenic assay

Cells were plated ~4 h prior to irradiation in order to ensure adherence to the bottom of the well plates in a monolayer. Cells were seeded at densities ranging from 100 to 40 000 cells depending on radiation dose and cell type. Plates were then mock irradiated or exposed to $^{137}$Cs or 6 MV X-rays and returned to the incubator for 7–14 days, depending on cell type. Twenty-four hours post radiation, additional media was added to each of the wells. Colonies were fixed, stained and manually counted. Colonies were only counted if there were >50 cells. Cell survival for each dose point was calculated based on the plating efficiency of the control (0 Gy) plates, and the data was fit using the Linear Quadratic (LQ) model:

$$-\ln S = \alpha D + \beta D^2, \tag{2}$$

where $S$ is the surviving fraction for a given dose, and $D$, $\alpha$ and $\beta$ are fit parameters determined by fitting the model to the data. This fit was obtained with MINUIT (CERN, Geneva, Switzerland), a numerical minimization program, using Chi-squared non-linear regression to solve for $\alpha$ and $\beta$. The RBE was then calculated by comparing the physical dose for $^{137}$Cs and 6 MV X-rays that result in 50% and 10% cell survival, as shown in Equation 1. Additionally, an RBE value was calculated directly from the data by taking the ratio of the surviving fractions after a dose of 2 Gy:

$$\mathrm{RBE}_{2\,Gy} = S_{\mathrm{control}}/S_{\mathrm{test}} \tag{3}$$

The uncertainty in RBE was calculated taking the standard deviation for both $\alpha$ and $\beta$ and propagating through the LQ model:

$$\sigma_y^2 = \sigma_\alpha^2\left(\frac{\partial y}{\partial \alpha}\right)^2 + \sigma_\beta^2\left(\frac{\partial y}{\partial \beta}\right)^2 + 2\left(\frac{\partial y}{\partial \alpha}\right)\left(\frac{\partial y}{\partial \beta}\right)\sigma_{\alpha\beta} \tag{4}$$

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### $^{137}$Cs irradiation

Irradiation was carried out using a $^{137}$Cs source irradiator (JL Shepherd and Associates, San Fernando, CA, USA) with a dose rate of 413 cGy min$^{-1}$. Dosimetry was carried out with EBT3 Gafchromic film (Ashland, Bridgewater, NJ, USA) by placing film squares in each well of a six-well plate (Corning, NY, USA) and repeating measurements three separate times. Irradiations were performed in six-well plates placed on top of a rotating platform for uniform irradiation. Dosimetry was confirmed for each experiment by placing film in each plate. A calibration curve was created using 6 MV X-rays, and all reference films were also exposed to known 6 MV X-ray doses to adjust the curve based on daily fluctuations. EBT3 has negligible energy dependence down to the kilovolt energy range; therefore, the application of a 6 MV X-ray calibration curve to films exposed to $^{137}$Cs irradiation is appropriate [14].

### 6 MV X-ray irradiation

X-ray irradiation was carried out using a Truebeam linear accelerator (Varian Medical Systems, Palo Alto, CA, USA) at a dose rate of 600 cGy min$^{-1}$ and energy of 6 MV. Prior to each cell irradiation, the output of the machine was measured using a calibrated Farmer ionization chamber. Dosimetry also included the use of EBT3 Gafchromic film by placing film squares in the three wells adjacent to the three wells with cells. Cells were irradiated with an anterior beam at 10 cm depth of water-equivalent material, where the radiation field is most uniform, with a field size of 20 cm × 20 cm. The six-well plates holding cells were immobilized in the acrylic jig shown in Fig. 1 to ensure a reproducible set-up for all experiments.

Eight radiation doses were delivered (0, 1, 2, 4, 5, 6, 8 and 10 Gy) to each appropriately seeded plate with the 0 Gy plate undergoing the same set-up for sham irradiation. Each plate

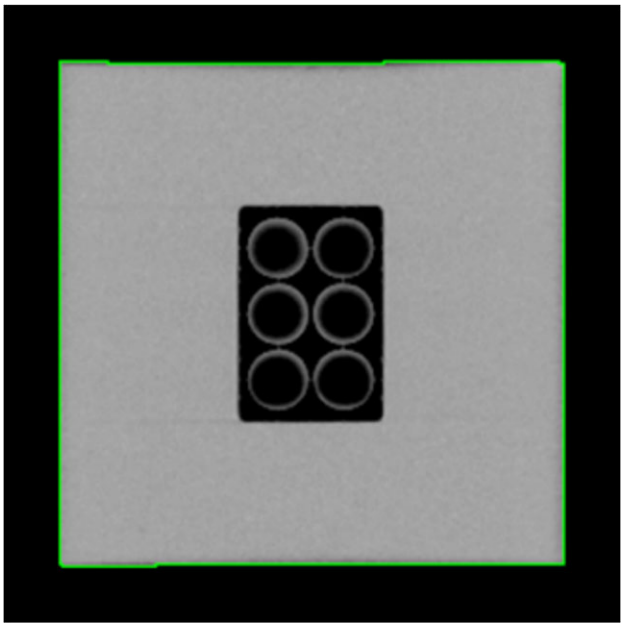

**Fig. 1. Cell irradiation set-up. CT image of 6 MV X-ray set-up; coronal slice (beam's eye view) shown. The plate of cells is in an acrylic jig for reproducible positioning, and has solid water surrounding it for uniform X-ray fluence.**

consisted of triplicate wells, and a total of three independent runs were conducted. Five cell lines were investigated with a total of 240 measurements taken to complete this study.

## RESULTS

### $^{137}$Cs and 6 MV X-ray dosimetry

Based on film dosimetry for the $^{137}$Cs irradiator, three corner wells were identified as being the most uniform across multiple runs. The $^{137}$Cs doses delivered for all experiments were found to be within ±5% of the intended dose. The uniformity of the radiation field generated by the linear accelerator was tested by placing EBT3 film in each of the six wells and was found to be within 1% across the entire plate. The 6 MV X-ray dose was found to be within ±3% for all dose points and experiments.

### Cell survival curves

The cell survival curves, using both $^{137}$Cs and 6 MV X-rays, for CHO, A549, U25, T98 and U2OS cells are shown in Fig. 2A–E. The fit parameters for the LQ model are listed in Table 1, along with the RBE values at 50% and 10% survival and the RBE value after 2 Gy was delivered. The RBE values for all cell lines ranged from 0.64 to 1.08 at 50% (1–3 Gy) survival, from 0.80 to 1.02 for 10% (3–7 Gy) survival and from 0.66 to 1.07 after a dose of 2 Gy. Additionally, the RBE values for CHO, A549 and T98 cell lines were all within 15% of 1.00 for all survival levels, indicating that the physical dose is comparable with the biological damage for these two types of radiation in these cell lines. Figure 3 shows the survival curves for all cell lines irradiated with $^{137}$Cs (A) and 6 MV X-rays (B). The spread between these curves shows the differences in radiation response, or $\alpha/\beta$ values, between cell lines.

## DISCUSSION

The RBE is an important parameter to consider in the treatment of patients as it directly affects the resulting biological damage from radiation. The purpose of this investigation was to characterize the RBE of $^{137}$Cs γ-rays in relation to 6 MV X-rays for various types of human cancer cells. These two types of radiation were chosen for this investigation due to the prevalent use of $^{137}$Cs in radiobiology research and the dominant use of 6 MV X-rays in clinical treatment, with our clinical standard serving as the reference radiation. A better understanding of the relationship between these two radiation types may have implications for future translational research.

Clonogenic assays were conducted with both types of radiation for five cell lines in order to evaluate the biological dose response. The CHO cell line was chosen as a historical line to compare with studies done previously. The A549, U251, T98 and U2OS human tumor cell lines serve as *in vitro* models of human tumors that have not been studied previously for determination of RBE. By studying five cell lines, four of which were human cancer lines, we were able to evaluate the RBE variation across cell lines. Further, these cell lines have different $\alpha/\beta$ values and therefore distinct radiation responses. These differences were confirmed and are shown in the survival curves in Fig. 3A and B. RBE values were calculated at 50% and 10% cell survival and after a single dose of 2 Gy. We chose to include $RBE_{2\ Gy}$ as it is a direct calculation of RBE from the data and does not depend on the parameters of the fit.

Three of the five cell lines (CHO, A549 and T98) resulted in RBE values as expected, close to 1.00. This indicates that the physical and biological dose is equivalent for these cell lines. Alternately, U251 and U2OS cells did not exhibit an RBE of 1.00. The U2OS cell line, an osteosarcoma cell line, is thought to be radiation resistant as it is clinically seen in patients with these tumors. However, our results showed a greater sensitivity to radiation because survival decreased more rapidly at lower doses compared with the other lines. This radiation sensitivity was also seen in the high alpha values as compared with the CHO, A549 and T98 cell data. Similar patterns were also seen with U251 cells, for which gliomas are known to be radiation resistant, but the survival data indicated decreased cell survival at lower doses. These discrepancies could be due to inherent characteristics of the U251 and U2OS cell lines and their response at higher doses (8–10 Gy) causing issues with quantification of survival. Due to this behavior, the error reported for these RBE values is quite large. Therefore, it remains to be seen whether the deviation from RBE values of 1.00 for the U251 and U2OS cell lines is a true radiation response or simply due to the behavior of these cells *in vitro*.

The error reported for RBE in this study was calculated by propagating one standard deviation for $\alpha$ and $\beta$ values from the LQ fit model. The majority of previous RBE data for X- and γ-rays report error on $\alpha$ and $\beta$ values only. As seen in Table 1, the error for RBE of $^{137}$Cs with respect to 6 MV X-rays for CHO, A549 and T98 cells ranges from 3 to 34%. Although this range of error seems large, the error reported for $\alpha$ and $\beta$ values is comparable with that

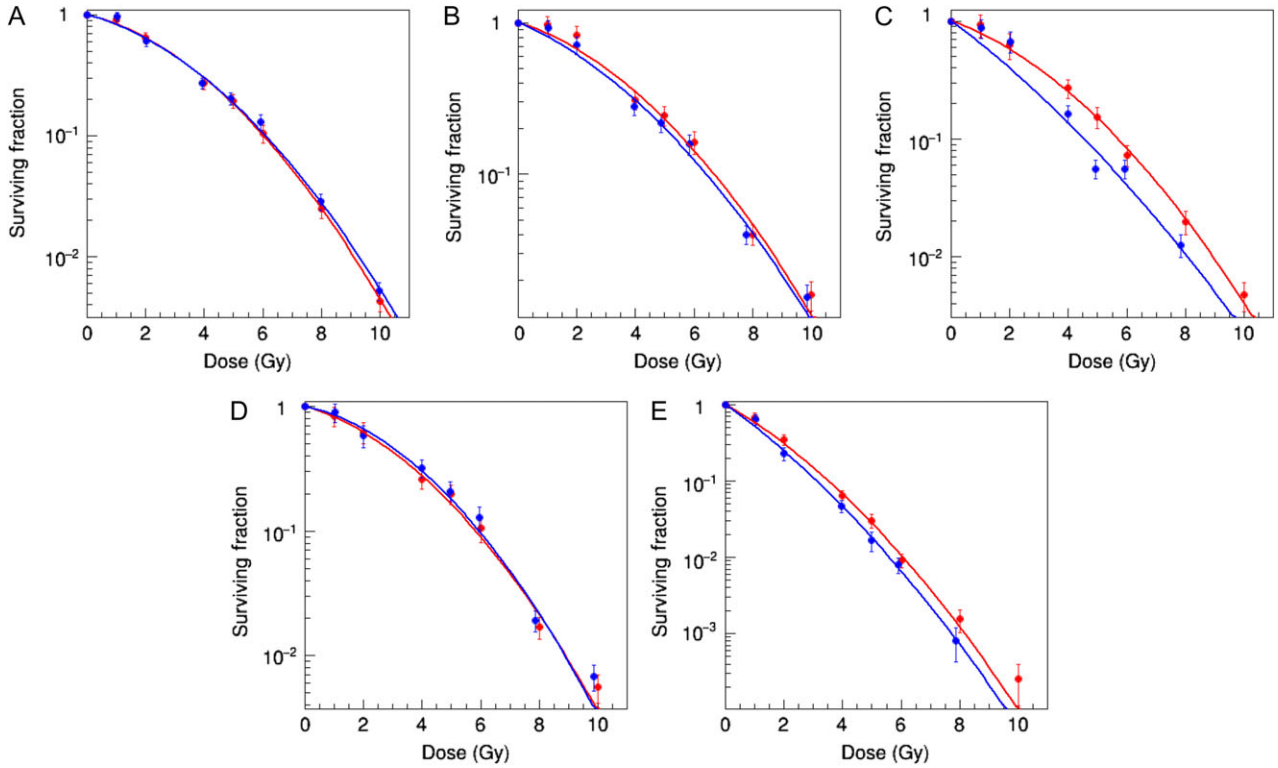


**Fig. 2. Cell survival curves comparing radiation with $^{137}$Cs (red) and 6 MV X-rays (blue) are shown for (A) CHO, (B) A549, (C) U251, (D) T98 and (E) U2OS cells. Circles denote the mean survival at each dose point, and error bars indicate the standard deviation.**

**Table 1. Fit parameters and RBE values for 50% and 10% cell survival after 2 Gy for all cell lines**

| Cell line | $\alpha$ (Gy$^{-1}$) | | $\beta$ (Gy$^{-2}$) | | $RBE_{0.5}$ | $RBE_{0.1}$ | $RBE_{2\ Gy}$ |
|---|---|---|---|---|---|---|---|
| | $^{137}$Cs | 6 MV X-rays | $^{137}$Cs | 6 MV X-rays | | | |
| CHO | 0.140 (±0.029) | 0.151 (±0.027) | 0.040 (±0.004) | 0.037 (±0.004) | 0.99 (±0.08) | 1.01 (±0.03) | 0.94 (±0.13) |
| A549 | 0.143 (±0.041) | 0.191 (±0.038) | 0.030 (±0.006) | 0.026 (±0.005) | 0.89 (±0.10) | 0.96 (±0.03) | 0.86 (±0.17) |
| U251 | 0.210 (±0.055) | 0.425 (±0.047) | 0.034 (±0.008) | 0.018 (±0.007) | 0.64 (±0.07) | 0.80 (±0.06) | 1.07 (±0.34) |
| T98 | 0.161 (±0.055) | 0.119 (±0.057) | 0.040 (±0.008) | 0.045 (±0.008) | 1.08 (±0.15) | 1.02 (±0.05) | 0.94 (±0.26) |
| U2OS | 0.510 (±0.074) | 0.632 (±0.074) | 0.041 (±0.013) | 0.034 (±0.013) | 0.84 (±0.11) | 0.89 (±0.05) | 0.66 (±0.16) |

$RBE_{0.5}$ = RBE at 50% cell survival, $RBE_{0.1}$ = RBE at 10% cell survival, $RBE_{2\ Gy}$ = RBE for cell survival after 2 Gy. RBE, $\alpha$ and $\beta$ values are all reported as mean ± standard deviation. Reference radiation is 6 MV X-rays.

of previous studies, and therefore the overall RBE error in this study is reasonable [15–18].

Comparable results were shown in the *in vivo* study by Fu *et al.* investigating the RBE of mouse jejunal crypt cells for various types of low- and high-LET radiation [12]. Although the published RBE values in the Fu *et al.* paper use $^{60}$Co as reference radiation, one can infer an RBE between $^{137}$Cs and 4 MV X-rays in single doses to be 1.03. The 4 MV and 6 MV beams are comparable, as their dominant interaction with matter is the same, and therefore this result agrees well with our RBE values for $^{137}$Cs and 6 MV X-rays. However, one limitation of that study was the focus on a single cell type in an animal model. Our study quantified RBE values across multiple human cell lines.

Though there are differences in LET, mean energy and dose rate between these two types of radiation, the RBE values measured in this study suggest the biological response is equivalent *in vitro*. As shown in the results from the U251 and U2OS cells, an alternative end point may need to be investigated in order to state with confidence whether the RBE for $^{137}$Cs and 6 MV X-ray radiation is 1.00,

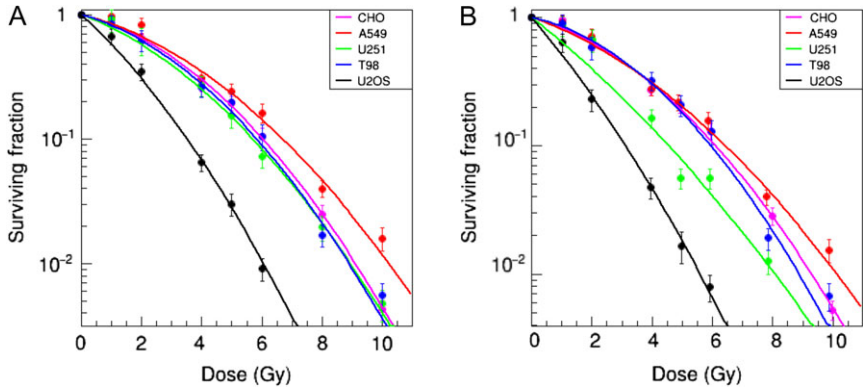


**Fig. 3. Cell survival curves for all cell lines irradiated with (A) $^{137}$Cs and (B) 6 MV X-rays. Circles denote the mean survival at each dose point, and error bars indicate the standard deviation.**

as the clonogenic assay may not be adequate. Additionally, our results from CHO, A549 and T98 cells show fluctuations in RBE values away from 1.00, depending on the level of survival specified. Therefore, this study demonstrates the difficulty of characterizing a single RBE value for all cell lines using a given type of radiation.

In conclusion, this study is the first to compare the RBE between $^{137}$Cs and 6 MV X-rays for human cancer cell lines. In order to determine the relationship between physical dose and biological effect, the end point of cell survival was quantified using the clonogenic assay. Five cell lines were evaluated in this study (CHO, A549, U251, T98 and U2OS), three of which had RBE values close to 1.00, ranging from 0.96–1.02 for 10% survival. The remaining two cell lines resulted in RBE values with greater variation from 1.00. The results presented in this work show the difficulty in characterizing RBE for different radiation types.

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## CONFLICT OF INTEREST

None.

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