Physics in Medicine & Biology

IPEM Institute of Physics and Engineering in Medicine

PAPER • OPEN ACCESS

The experimental dose ranges influence the LET_d dependency of the proton minimum RBE (RBE_{min})

To cite this article: Eivind Rørvik *et al* 2019 *Phys. Med. Biol.* **64** 195001

IOP Publishing Phys. Med. Biol. 64 (2019) 195001 (9pp) https://doi.org/10.1088/1361-6560/ab369a

Physics in Medicine & Biology

IPEM Institute of Physics and Engineering in Medicine

OPEN ACCESS

PAPER

The experimental dose ranges influence the LET_d dependency of the proton minimum RBE (RBE_{min})

Eivind Rørvik[1,3], **Sara Thörnqvist**[1,2] **and Kristian S Ytre-Hauge**[1]

[1] Department of Physics and Technology, University of Bergen, Bergen, Norway
[2] Department of Oncology and Medical Physics, Haukeland University Hospital, Bergen, Norway
[3] Author to whom any correspondence should be addressed.

E-mail: eivind.rorvik@uib.no

Keywords: proton therapy, relative biological effectiveness, proton cell experiments, linear quadratic model, linear energy transfer

RECEIVED
11 January 2019

REVISED
21 July 2019

ACCEPTED FOR PUBLICATION
29 July 2019

PUBLISHED
23 September 2019

Abstract

Cell experiments have shown the proton relative biological effectiveness (RBE) to vary with dose and linear energy transfer (LET), which has led to development of variable RBE models. The RBE is normally estimated from two independent functions, the RBE_{max} and RBE_{min}, describing the extreme RBE at low and high doses. While there is consensus that RBE_{max} increases with increasing LET, the RBE_{min} is not uniformly defined and its dependency on LET is deviating. In this work, we analysed this dependency and its sensitivity to variations of the experimental dose range.

We performed a literature search to find data from existing monoenergetic proton cell survival experiments with $(\alpha/\beta)_x$ values below 5 Gy and dose averaged LET (LET_d) values below 20 keV μm^{-1}. From the experiments the doses and their corresponding survival data were extracted. Based on these data, multiple restricted databases were generated by sequential exclusion of low dose data in the experiments followed by a linear-quadratic (LQ) fit. The quadratic component from the LQ-fit was used to estimate RBE_{min}. The LET_d dependency of RBE_{min} was determined by fitting a linear function to the RBE_{min} values estimated from the restricted databases.

Our analysis showed the LET_d dependency of RBE_{min} to be significantly influenced by the experimental dose range. By including experiments with doses below 1 Gy in the database, we found that RBE_{min} increased with increasing LET_d. By excluding the low dose experiments in our database, the RBE_{min} became constant for all LET_d values. For an LET_d value of 5 keV μm^{-1}, a restricted database including the data with the lowest doses gave an RBE_{min} of 1.4 ± 0.1, while databases with only high dose data (>2 Gy) gave an RBE_{min} of 1.0 ± 0.1. None of our restricted databases gave a decreasing RBE_{min} with increasing LET_d.

Our study showed that RBE_{min} has a small yet significant dependency on LET_d for tissues with low $(\alpha/\beta)_x$ ratio. The LET_d dependency of RBE_{min} varied substantially with the experimental dose range. Including experiments with high minimum dose in RBE models may lead to underestimation of the RBE.

1. Introduction

Protons can more effectively produce biological damage as compared to photons, due to differences in the ionization processes between the radiation types (Tommasino and Durante 2015). Comparison of proton and photon treatment plans by the physical dose is therefore not enough. A conversion factor, the relative biological effectiveness (RBE), has been introduced in proton therapy to enable transferring of treatment protocols and experience gathered from γ- and x-ray irradiation (RBE Committee 1963). Clinical proton therapy applies a constant RBE of 1.1, even though the RBE is known to vary with dose, radiation quality and tissue type. These parameters are included in most phenomenological RBE-models for proton therapy, i.e. models based on results from proton beam *in vitro* experiments (Paganetti 2014).

The phenomenological RBE-models are typically based on the linear quadratic model (LQ-model), which model the dose-response by quantifying the α_p and β_p parameters (Rørvik *et al* 2018). The RBE at its maximum

and minimum can bea RBE model is then functions described by the variables RBE_{max} ($=\alpha_p/\alpha_x$) and RBE_{min} ($=\sqrt{\beta_p/\beta_x}$) (Schuemann *et al* 2018). These functions can be dependent on the radiation quality and tissue type, normally represented by the dose-averaged linear energy transfer (LET_d) and the fractionation sensitivity of the photon radiation ($(\alpha/\beta)_x$), respectively.

The RBE-models agree on an increasing RBE_{max} with increasing LET_d, but the dependency of RBE_{min} on LET_d differ (Rørvik *et al* 2018). A recent review showed that 7 out of 12 phenomenological models assume a constant RBE_{min} (equal to one) for all LET_d values. However, in the models by Carabe *et al* (2012), Jones (2015) and Peeler (2016) the RBE_{min} increased with increasing LET_d value, which is in contrast to the models by Belli *et al* (1997) and McNamara *et al* (2015), where the RBE_{min} decreased with increasing LET_d (Rørvik *et al* 2018). The RBE_{min} relationships of the analytical biophysical models are also deviating, as shown by Manganaro *et al* (2017). According to their calculations, the repair–misrepair fixation (RMF) model, the microdosimetric-kinetic model (MKM) and the local effect model (LEM) predict an increasing, a constant and a decreasing relationship with LET, respectively. The differences in the phenomenological models mainly arise from the variations in the experimental data, as the models' assumptions are freely fitted to the experimental database. Even though all models are based on cell survival experiments, the experiments are not performed in a consistent manner between the experimental groups. They differ both in regard to the experimental setup such as dose range, dose rate and energy spectra and the analysis of the experimental data e.g. regression technique and uncertainty calculations.

A large amount of *in vitro* data exists for protons, which could be used to investigate different dependencies of the RBE_{min} functions. The *in vitro* data consist of multiple experiments of cell survival from various dose ranges, at a certain LET_d value, and from which LQ-model parameters are extracted. The experimental setup varies between the different experiments, including dose range, as well as the regression method to determine the α_p and β_p parameters from the cell survival data. The fitted LQ-model parameters are highly dependent of the number of data points and the dose range of these points, as shown by Garcia *et al* (2006) for cells irradiated with photon radiation. Their analysis indicated that including data points with low doses increased the β_p parameter, as compared to when the regression was restricted to only high doses.

In this study we therefore analysed how the LET_d dependency of RBE_{min} is influenced by the dose range of the proton *in vitro* experiments. We aimed to determine whether the common assumption of a constant RBE_{min} for all LET_d values holds independently of dose range.

2. Materials and methods

We performed a literature search to find all survival experiments of cells irradiated *in vitro* by external proton beam therapy (per July 2018). In our work, an experiment was defined as a series of cell survival data from proton irradiations at different dose levels, but with a unique $(\alpha/\beta)_x$ value and a single LET_d. Consequently, each experiment represents a unique cell survival curve, which can be parameterised by fitting the LQ-model to the experimental data points.

We only included experiments executed with monoenergetic beams, as a narrow LET spectra give a better description of the LET range of interest compared to beam with broad LET and energy distributions (Dahle *et al* 2017). We excluded experiments with LET_d above 20 keV μm^{-1} from our database due to its limited relevance in clinical treatments. We further restricted our database to cell experiments where the reference experiment had an $(\alpha/\beta)_x$ value below 5 Gy. The latter limit the response from early reacting cells with high $(\alpha/\beta)_x$ values, as our database then represented late responding tissue. By only focusing on a narrow range of $(\alpha/\beta)_x$ values, we avoided the general $(\alpha/\beta)_x$ dependency and potentially reduced the data noise. Other established models have included diverse definitions of the $(\alpha/\beta)_x$ dependency (Carabe *et al* 2012, McNamara *et al* 2015, Peeler 2016).

From each experiment, data points were extracted with WebPlotDigitizer (Rohatgi 2018) from the survival curve figures. The software was used to calibrate the axes and calculate the dose and survival of each individual data point from all experiments. The extraction was restricted to dose versus survival values, omitting potentially reported uncertainty bounds as the uncertainty of the data points were not reported in a concise manner and also lacking in some studies. The LQ-model was subsequently fitted to each extracted proton experiment in MATLAB using linear unweighted regression to the logarithmic survival data, as shown by the LQ-model equations:

$$\ln\left(\frac{S}{S_0}\right) = -\alpha_p D_p - \beta_p D_p^2, \tag{1}$$

where D_p is the physical dose deposited by protons per fraction, $\frac{S}{S_0}$ is the survival fraction of the radiated cells compared to the reference cells which are not irradiated and α_p and β_p are the radiosensitivity parameters of the LQ-model found by the regression. The regression technique was independent of the method applied by the study from which the data was extracted. The obtained LQ-model parameters were therefore compared to the reported values (figure A1). We used the reported LQ-model parameters of the photon experiments, as the data points of the reference experiments were not included in all publications, rendering the refitting of the LQ-model

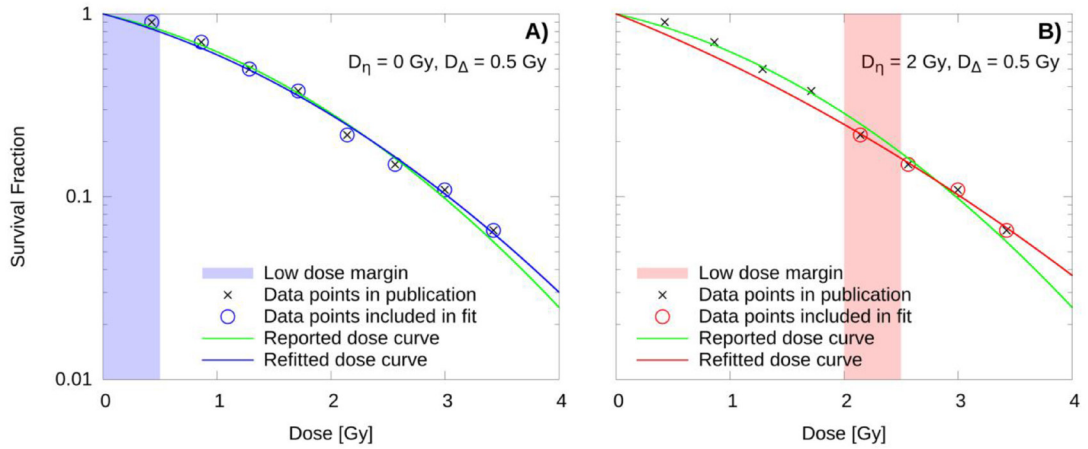

Figure 1. Cell survival curves of an experiment with data points extracted from Guan *et al* (2015). The shaded area is defined by the interval of D_η and $D_\eta + D_\Delta$. Both figures have an identical D_Δ of 0.5 Gy but different D_η of 0 Gy (example A) and 2 Gy (example B). The figure illustrates how only data points with a dose value greater or equal to D_η and with a data point located in the shaded area are included in the refit, as indicated by the coloured circles around the crosses. If the margin, D_Δ, was set smaller, the experiment in example A would have failed the inclusion criteria and consequently not been accepted in the restricted database.

difficult (Folkard *et al* 1989, Prise *et al* 1990, Schettino *et al* 2001). From the LQ-model parameters, we found the $\mathrm{RBE}_{\mathrm{min}}$ of the experiment by:

$$\mathrm{RBE}_{\mathrm{min}} = \sqrt{\beta_p/\beta_x}, \tag{2}$$

where β_p is our new fitted parameter to the proton data and the β_x is the reported parameter of the photon data (Carabe-Fernandez *et al* 2007). The uncertainties in the $\mathrm{RBE}_{\mathrm{min}}$ values were found with the Gauss error propagation principle:

$$\Delta\mathrm{RBE}_{\mathrm{min}} = \frac{1}{\sqrt{2}\beta_x}\sqrt{\frac{\beta_x}{\beta_p}\Delta\beta_p^2 + \frac{\beta_p}{\beta_x}\Delta\beta_x^2}. \tag{3}$$

Further, to investigate the dependence of the $\mathrm{RBE}_{\mathrm{min}}$ on the dose range of the experiments, we refitted the LQ-model to multiple subsets of the data points of each experiment by introducing two new variables. The first dose variable, D_η, is the minimum dose cut-off such that experimental data points lower than this cut-off were excluded. The second variable, D_Δ, described a margin above D_η such that the experiment must have a dose point in the interval of D_η and $D_\eta + D_\Delta$ to be refitted to the LQ-model. The application of two different D_η but identical D_Δ is shown in figure 1. The $\mathrm{RBE}_{\mathrm{min}}$ derived with equation (2) using the refitted β_p-parameter from those experiments fulfilling the inclusion variables were used to construct a restricted database. To analyse the implication of low dose data (or more importantly, the lack of this data) on the $\mathrm{RBE}_{\mathrm{min}}$ function, multiple restricted databases were created by varying the D_η from 0 to 2.5 Gy and varying D_Δ from 0.2 to 0.7 Gy. The upper level of 2.5 Gy was motivated both by the limited amount of available data for higher D_η and the most common fraction doses applied in proton therapy.

The $\mathrm{RBE}_{\mathrm{min}}$ values were compiled in the restricted databases with the $\mathrm{LET}_{\mathrm{d}}$ values of the experiments. Based on the restricted databases, the simplest form for $\mathrm{LET}_{\mathrm{d}}$ dependency was explored by the following equation:

$$\mathrm{RBE}_{\mathrm{min}} = 1 + \mathrm{cLET}_{\mathrm{d}}, \tag{4}$$

where c is a constant determined from linear regression. Two regression methods were used, as the c value was determined with both unweighted and weighted regression. The inverse of the uncertainty, calculated by equation (3), was used as weights for the weighted regression. With this relationship, a c-value significantly different from 0 indicates that $\mathrm{RBE}_{\mathrm{min}}$ has a $\mathrm{LET}_{\mathrm{d}}$ dependency. We determined the first order c coefficient and its uncertainty by both unweighted and weighted regression.

3. Results

From our literature search, we found 76 experiments, each with a specific survival and dose value and a corresponding $\mathrm{LET}_{\mathrm{d}}$, fulfilling our inclusion criteria (Hei *et al* 1988, Folkard *et al* 1989, 1996, Prise *et al* 1990, Belli *et al* 1993, 1998, Green *et al* 2001, 2002, Schettino *et al* 2001, Baggio *et al* 2002, Schuff *et al* 2002, Chaudhary *et al* 2014, Guan *et al* 2015, Patel *et al* 2017, Howard *et al* 2018). This corresponded to in total 529 data points,

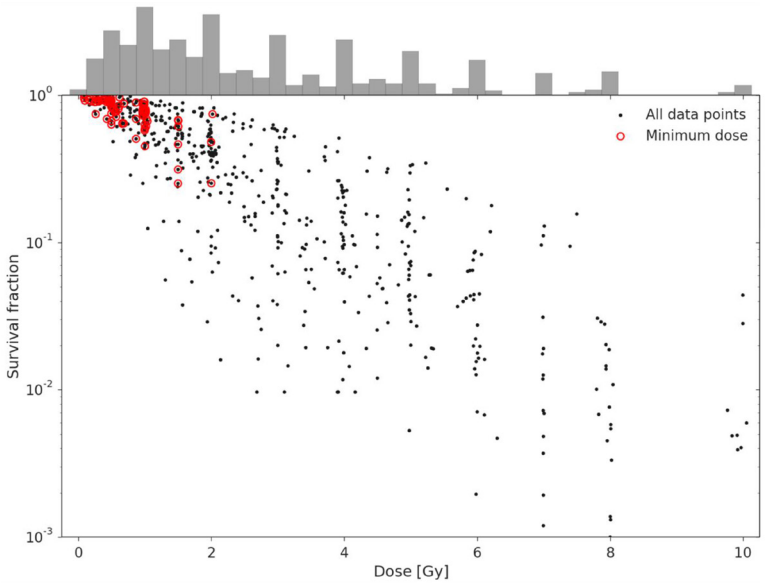

Figure 2. A scatterplot with a corresponding histogram of the data from all experiments fulfilling the criteria of our literature search. The red points indicate the minimum dose of each experiment.

Table 1. The mean values of the LQ-model parameters of the included experiments. The mean values for the restricted databases were found from varying D_η but with constant margin, D_Δ, of 0.5 Gy.

D_η [Gy]	Mean α_p [Gy^{-1}]	Mean β_p [Gy^{-2}]	α_p/β_p [Gy]	Number of experiments in database	Mean lowest dose [Gy]
0.0	0.24	0.20	1.18	34	0.33
0.5	0.28	0.14	2.02	61	0.77
1.0	0.29	0.15	1.94	49	1.20
1.5	0.28	0.09	2.97	58	1.78
2.0	0.30	0.08	3.61	29	2.15
2.5	0.25	0.08	2.85	22	2.80

which are shown in figure 2. As seen by the histogram in this figure, the data points of the experiments are mainly clustered around integer numbers of Gy and within the dose range of 0.1–10.1 Gy.

The originally reported values of α_p and β_p and those extracted from our refitting procedure using unweighted regression to the logarithmic data of cell survival are shown in figure A1. The absolute difference between the reported data and refitted data was on average 0.05 ($\pm$0.07) Gy^{-1} for α_p and 0.02 ($\pm$0.04) Gy^{-2} for the β_p value. In figure 3, the distribution of the $(\alpha/\beta)_x$ and $\mathrm{LET_d}$ values of the original (unrestricted) database is shown. As seen, the majority of the cell lines have an $(\alpha/\beta)_x$ of 1–4 Gy and the database is dominated by low $\mathrm{LET_d}$ experiments.

The restricted databases from varying D_η, but keeping D_Δ constant (0.5 Gy), is shown in table 1. In general, the number of included experiments was greatest around D_η of 0.5 to 1.5 Gy. The effect on varying the margin D_Δ is shown in table A1, where the number of experiments included in the databases grow as D_Δ increased. All experiments included in the restricted database have the lowest dose value between D_η and $D_\eta + D_\Delta$. By rising either of the two parameters, the mean value of the minimum dose value of the included experiment is naturally increased, as shown by the table A2.

In table 1 and also illustrated by the figure 1, increasing the D_η resulted in a reduction, and thereby potential underestimation, of the β_p parameter. In the example, where a constant D_Δ is applied (0.5 Gy), the β_p parameter is reduced by more than 50% for D_η of 1.5 Gy or higher as compared to β_p from the unrestricted experiment. The β_p parameter is relatively stable for D_η values above 1.5 Gy. Most experiments are refitted if a D_η value between 0.5 Gy and 1.5 Gy is applied. The implications for α_p are smaller and lacking a clear trend.

From the refitted α_p and β_p parameters the $\mathrm{RBE_{min}}$ value is found with its uncertainty for every experiment included in our databases. This is exemplified for three restricted databases with a different number of included experiments (figure 4(A) include 34 experiments, figure 4(B) 49 experiments and figure 4(C) 29 experiments). Applying linear regression according to equation (4), we find the regression coefficient, c to be small but positive for all D_η values. In addition, the c value is decreasing with increasing D_η, which also can be seen in figure 5. Comparing the $\mathrm{RBE_{min}}$ values found with unweighted regression to the databases with $D_\Delta = 0.5$ Gy, we find that a function derived from a database with $D_\eta = 0$ Gy will calculate an $\mathrm{RBE_{min}}$ value between 1.4 ± 0.1 and 2.5 ± 0.2

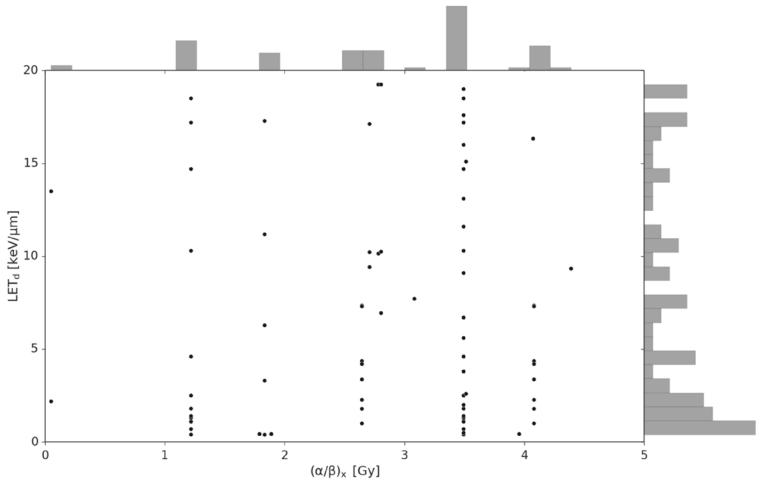

Figure 3. The $(\alpha/\beta)_x$ and LET_d values of all included experiments, with the corresponding histograms of the total distribution.

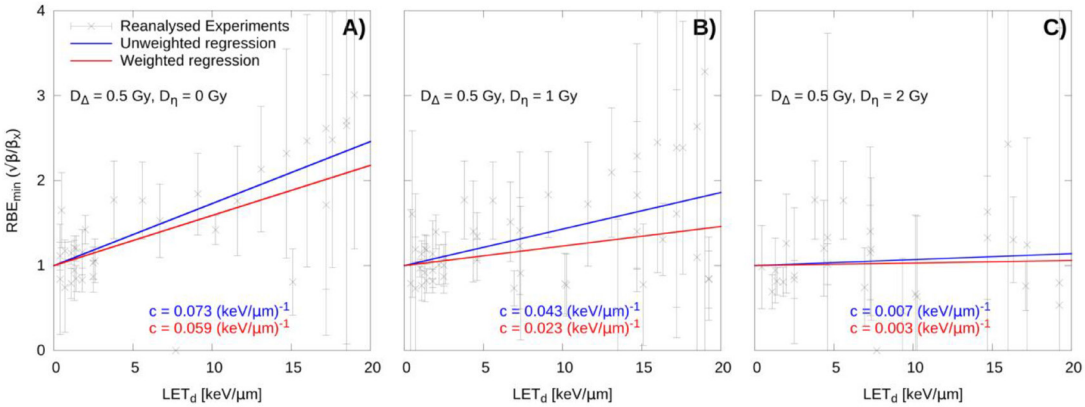

Figure 4. The RBE_{min} values of the experiments in the restricted databases with $D_\Delta = 0.5$ Gy and three different values for D_η: 0 Gy (A), 1 Gy (B) and 2 Gy (C).

in the LET_d range from 5 to 20 keV μm^{-1}. These values are significantly smaller for a function derived from a database with $D_\eta = 2$ Gy, as the same LET_d range will estimate RBE_{min} values between 1.0 ± 0.1 and 1.1 ± 0.2. Hence, if experiments executed with a higher minimum dose was used for estimating RBE_{min} the LET dependency was diminished.

Furthermore, unweighted regression consistently resulted in a stronger dependency on LET_d for RBE_{min}. A c value equal to 0 corresponds to a constant RBE_{min} independent of LET_d. For unweighted regression this required a $D_\eta > 2.0$ Gy in combination with a large D_Δ and for weighted regression a $D_\eta > 1.0$ Gy in combination with as small D_Δ. Hence, even for weighted regression and inclusion of doses less than 1 Gy, the results indicate the RBE_{min} to be significantly higher than the commonly assumed 1 for high LET_d values.

4. Discussion

Our study revealed a clear correlation between the range of doses included in experiments and the variability of RBE_{min} with LET_d. Only if data from low doses are excluded from the modelling, the assumption that the RBE_{min} is constant for all with LET_d values appears reasonable. When including doses of 1 Gy or less than, our analysis shows a small, but significant, increase in RBE_{min} with increasing LET_d. Experiments consisting of data points only with high doses could therefore estimate a lower β_p parameter compared to experiments including low dose data. This indicates that RBE models which include experiments with high minimum doses could underestimate the RBE.

The sensitivity of low doses for the estimation of the β_p parameter could contribute to the diverging results on the LET_d dependency for RBE_{min} in the literature. As an example, Guan *et al* (2015) found both the α_p- and β_p parameters to be nonlinearly increasing with increasing LET_d value. These experiments included low dose values, with a minimum dose below 0.5 Gy, as compared to other experiments. In contrast, Chaudhary *et al*

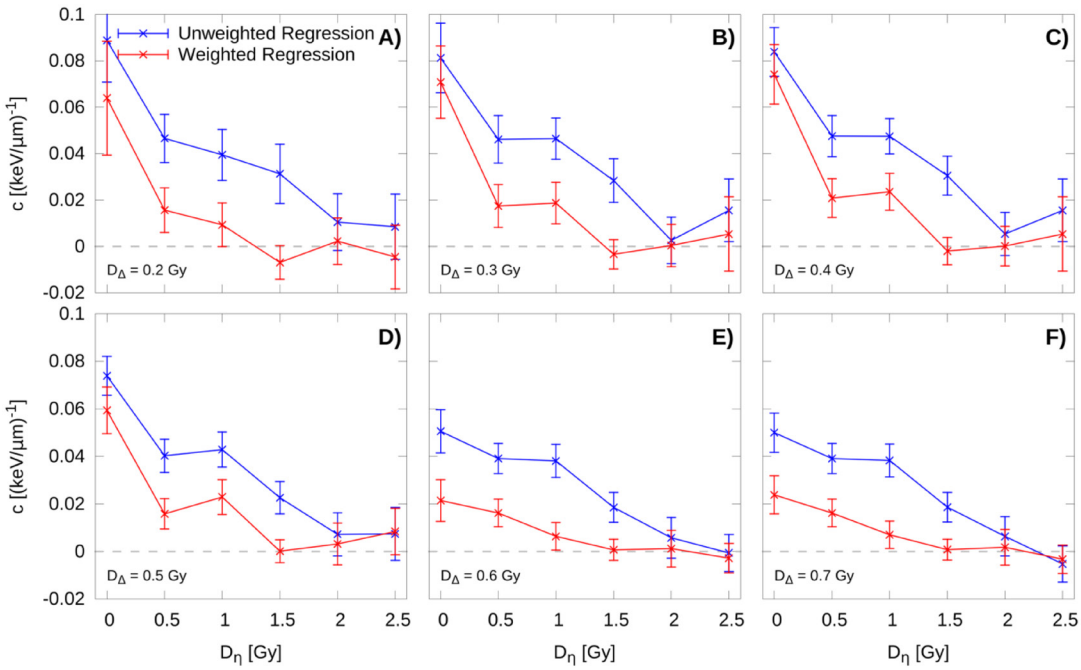

Figure 5. The variation of the regression coefficient, c as a function of D_η for various D_Δ values, increasing from subfigure A–F. The reported error bars show one standard deviation of the determination of c from regression.

(2014) found the β_p parameter to be decreasing with increasing LET_d value, which could be caused by their high the minimum dose of 1.5 Gy in the experiments, as indicated by the three red circles with at 1.5 Gy in figure 1. We hypothesize that the β_p values could have been higher, if the experiment included one or more data points with a dose value under 1.5 Gy. Based on our analysis, we therefore recommend that future experiments will be performed with low doses included in the experimental setup, with at least one data point with a dose maximum equal to 1 Gy, preferably lower.

For our analysis, we included the simplest definition of a variable RBE_{min}: Variation with a single parameter. However, we cannot exclude more a complex relationship between LET_d and RBE_{min}. The definition can be modified to abandon the assumption of RBE_{min} equals 1 at $LET_d = 0$, by determining the constant from the regression analysis. This was done in the models made by Carabe *et al* (2012), McNamara *et al* (2015) and Peeler (2016). Further, as previously shown for RBE_{min}, the LET relationship might also be non-linear (Peeler 2016). There is however a large variation in the existing data, as seen in figure 4, thus there is no clear non-linear trend. More experimental data and verification of existing models, e.g. the comparison of models to experimental data by Polster *et al* (2015) and Mairani *et al* (2017), needs to be done to determine which model that give the best representation of the clinical dose response.

Based on the analysis in this work, we recognise that models with an RBE_{min} function with a positive dependency of LET_d could estimate a more precise RBE and should be explored further. As shown in an earlier analysis of phenomenological models (Rørvik *et al* 2018), the use of a constant RBE_{min} is inherited from earlier models and not based on quantitative analysis of experimental data. Generally speaking, we recommend that new models should not adapt a constant definition of RBE_{min} without performing statistical analysis of the database. Calculation of the absolute effect of a dose dependent RBE_{min} on RBE estimates have not been performed, as mixing of model functions generally is not recommended.

We chose to focus our RBE_{min} analysis on late responding tissue by excluding cell lines with an $(\alpha/\beta)_x$ value above 5 Gy. This threshold was a compromise between focusing on the late responding tissue, but still having sufficient experiments ($N = 76$) for our regression analysis. Most models are consistent in their definition of the $(\alpha/\beta)_x$ dependency on RBE_{max}, with an inversely proportional RBE_{max} with increasing $(\alpha/\beta)_x$. However, the $(\alpha/\beta)_x$ dependency of the RBE_{min} is deviating in previously published models (Rørvik *et al* 2018). Carabe *et al* (2012) used the same inversely proportional definition for RBE_{min} as they did for RBE_{max}, however the experimental database only consisted of V-79 cells with a narrow $(\alpha/\beta)_x$ range around 2–3 Gy. McNamara *et al* (2015) assumed that RBE_{min} is increasing with the square root of $(\alpha/\beta)_x$, while Peeler assumed RBE_{min} to be linear dependent of the $(\alpha/\beta)_x$ value. We did not include an $(\alpha/\beta)_x$ dependency and omitted the issue altogether by analysing a database with a narrow $(\alpha/\beta)_x$ range, mainly between 1 and 4 Gy. We will therefore not postulate if the same effect will be seen for early responding tissue with high $(\alpha/\beta)_x$ values, however, our analysis is of interest in itself, as most organs at risk are regarded as late responding tissue with low $(\alpha/\beta)_x$ values.

We introduced a method in our analysis by applying a minimum dose with a margin to construct restricted databases from a major database of existing experiments. The refitting of the database with a low margin value, excludes many experiments from the regression analysis, as seen in table A1. The quality of the regression analysis may therefore be reduced as compared to regression performed on a database constructed with higher margin values. Therefore the found regression coefficients should be interpreted carefully due to the small amount of data. An effect of the low data amount can be seen in figure 5, where the c values fluctuates for D_Δ values up to 0.5 Gy, while the c values of a D_Δ of 0.6 Gy or 0.7 Gy monotonically decreases with increasing LET_d value. The fluctuations in the mean values are however smaller than the uncertainty, thus we can assume there is a steady decreasing trend for all D_Δ values. It should be mentioned that the points in figures 4(E) and (F) are based on overlapping databases, as the interval between the points (0.5 Gy) is smaller than the D_Δ. This will smooth out any oscillations in the dataset. We found 0.5 Gy to be a reasonable size for D_Δ, as a compromise between having enough experiments in the restricted databases but still allowing for separation of the effect from an increasing D_η. This value of D_Δ is therefore used in the other examples shown in figures 1 and 4 and table 1.

We analysed and treated α_p and β_p as two independent parameters, which is a common practice for LQ-based RBE models (Rørvik *et al* 2018). This is however a major approximation, as a change in one of the parameters will be counteracted by the other parameter. The α_p parameter will in general increase when the β_p parameters decreases with increasing D_η value. An example of this can be seen by the initially steeper curve in figure 1(B) compared to the curve in figure 1(A). Additionally, there is a great variation in the regression fitting techniques used in the different experiments reported in the literature, which further impacts the reported LQ-model parameters. In an attempt to reduce variations from different regression techniques we refitted the LQ-model to the data values with a consistent method for all experiments. Abolfath *et al* (2017) also omitted this issue by making a model directly from data values. They used a 'global-fitting' method for the LQ-model parameters, by making a regression fit of the LET_d dependent α_p and β_p functions in one single regression, instead of treating them independently as we did. The 'global-fitting' method is novel and could potentially reproduce the actual response better, however, Abolfath *et al* (2017) only adapted the method to two cell lines, independent from each other. It is therefore not directly possible to compare the Abolfath model to our model functions in its current form. The method needs to be developed further to create a general model from multiple cell lines at once.

In a clinical scenario where a patient is treated with a normal fractionated plan, the practical dose range is in between 0 and 2–3 Gy. This is relevant for RBE models, as there should be good correspondence between the experimental data of the model and the clinical usage. Therefore, we advise that the dose region of the RBE models' background data should be considered relevant in the selection of RBE models. Future RBE models could acknowledge the importance of the dose range of the experiments and adapt inclusion criteria for the experimental database accordingly.

5. Conclusion

Based on a compilation of existing *in vitro* data with $(\alpha/\beta)_x$ values below 5 Gy, RBE_{min} was found dependent on LET_d. The small yet significant positive linear correlation of LET_d on RBE_{min} was visible for databases with minimum experimental doses of 1 Gy or below. If the minimum dose of the cell survival data was set greater than this value, the LET_d dependency was lost and RBE_{min} become constant. The inclusion of experiments with high minimum dose in phenomenological RBE models could therefore lead to underestimation of the actual proton RBE.

Acknowledgments

This work was funded by Bergen Research Foundation.

Appendix

The value and uncertainty of the α_p and β_p parameters of the different experiments are given in figure A1. The refitted values shown are found from a refit to all data points of the experiment, not only a restricted selection of data points with high dose values. The variation of the reported and refitted parameters arises from different fitting techniques, as we only used a linear regression technique. Some articles used the uncertainty in the data in a weighted regression technique. As our database is found from figures, not reported values, this can also explain the difference in figure A1.

By varying D_η and D_Δ values, the number of experiments included in the restricted will also vary, as shown in table A1. The number increases with the width of the dose margin (D_Δ) and is largest for D_η between 0.5 and 1.5 Gy. The variation of the parameters also varies the dose values of the included experiments. The actual mean minimum dose is given in table A1.

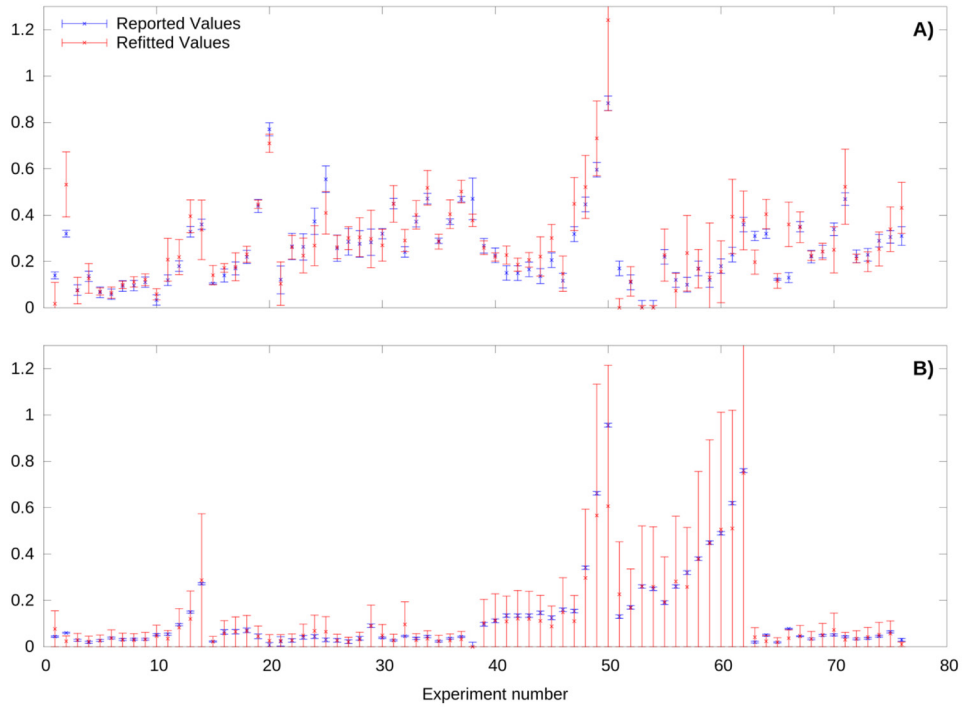

Figure A1. The α_p and β_p values of all proton cell irradiation experiments, with the reported values in blue, the refitted data in red.

Table A1. The number of experiments included in the regression of the RBE_{min} of the included experiments.

	D_Δ [Gy]					
D_η [Gy]	0.2	0.3	0.4	0.5	0.6	0.7
0.0	6	14	22	34	41	45
0.5	27	33	39	61	69	69
1.0	27	36	43	49	55	56
1.5	21	29	34	58	68	69
2.0	20	23	26	29	31	33
2.5	8	11	11	22	31	34

Table A2. The mean value of the actual minimum dose of the experiments included in the regression of the RBE_{min} of the included experiments.

	D_Δ [Gy]					
D_η [Gy]	0.2	0.3	0.4	0.5	0.6	0.7
0.0	0.13	0.21	0.26	0.33	0.36	0.39
0.5	0.59	0.61	0.65	0.77	0.80	0.80
1.0	1.07	1.12	1.16	1.20	1.23	1.24
1.5	1.6	1.62	1.66	1.78	1.82	1.82
2.0	2.06	2.08	2.11	2.15	2.18	2.21
2.5	2.60	2.64	2.64	2.80	2.87	2.89

ORCID iDs

Eivind Rørvik https://orcid.org/0000-0002-1794-9955
Kristian S Ytre-Hauge https://orcid.org/0000-0002-3597-1611

References

Abolfath R, Peeler C R, Newpower M, Bronk L, Grosshans D and Mohan R 2017 A model for relative biological effectiveness of therapeutic proton beams based on a global fit of cell survival data *Sci. Rep.* **7** 8340

Baggio L, Cavinato M, Cherubini R, Conzato M, Cucinotta F, Favaretto S, Gerardi S, Lora S, Stoppa P and Williams J R 2002 Relative biological effectiveness of light ions in human tumoural cell lines: role of protein p53 *Radiat. Prot. Dosim.* **99** 211–4
Belli M, Campa A and Ermolli I 1997 A semi-empirical approach to the evaluation of the relative biological effectiveness of therapeutic proton beams: the methodological framework *Radiat. Res.* **148** 592–8
Belli M *et al* 1998 RBE-LET relationships for cell inactivation and mutation induced by low energy protons in V79 cells: further results at the LNL facility *Int. J. Radiat. Biol.* **74** 501–9
Belli M, Cera F, Cherubini R, Haque A M, Ianzini F, Moschini G, Sapora O, Simone G, Tabocchini M A and Tiveron P 1993 Inactivation and mutation induction in V79 cells by low energy protons: re-evaluation of the results at the LNL facility *Int. J. Radiat. Biol.* **63** 331–7
Carabe A, Moteabbed M, Depauw N, Schuemann J and Paganetti H 2012 Range uncertainty in proton therapy due to variable biological effectiveness *Phys. Med. Biol.* **57** 1159
Carabe-Fernandez A, Dale R G and Jones B 2007 The incorporation of the concept of minimum RBE (RBEmin) into the linear-quadratic model and the potential for improved radiobiological analysis of high-LET treatments *Int. J. Radiat. Biol.* **83** 27–39
Chaudhary P *et al* 2014 Relative biological effectiveness variation along monoenergetic and modulated Bragg peaks of a 62 MeV therapeutic proton beam: a preclinical assessment *Int. J. Radiat. Oncol.* **90** 27–35
Dahle T J, Rykkelid A M, Stokkevåg C H, Mairani A, Görgen A, Edin N J, Rørvik E, Fjæra L F, Malinen E and Ytre-Hauge K S 2017 Monte Carlo simulations of a low energy proton beamline for radiobiological experiments *Acta Oncol.* **56** 779–86
Folkard M, Prise K M, Vojnovic B, Davies S, Roper M J and Michael B D 1989 The Irradiation of V79 Mammalian cells by protons with energies below 2 MeV: part I: experimental arrangement and measurements of cell survival *Int. J. Radiat. Biol.* **56** 221–37
Folkard M, Prise K M, Vojnovic B, Newman H C, Roper M J and Michael B D 1996 Inactivation of V79 cells by low-energy protons, deuterons and helium-3 ions *Int. J. Radiat. Biol.* **69** 729–38
Garcia L M, Leblanc J, Wilkins D and Raaphorst G P 2006 Fitting the linear–quadratic model to detailed data sets for different dose ranges *Phys. Med. Biol.* **51** 2813
Green L M, Murray D K, Tran D T, Bant A M, Kazarians G, Moyers M F and Nelson G A 2001 Response of thyroid follicular cells to gamma irradiation compared to proton irradiation. I. Initial characterization of DNA damage, micronucleus formation, apoptosis, cell survival, and cell cycle phase redistribution *Radiat. Res.* **155** 32–42
Green L M, Tran D T, Murray D K, Rightnar S S, Todd S and Nelson G A 2002 Response of thyroid follicular cells to gamma irradiation compared to proton irradiation: II. The role of connexin 32 *Radiat. Res.* **158** 475–85
Guan F *et al* 2015 Spatial mapping of the biologic effectiveness of scanned particle beams: towards biologically optimized particle therapy *Sci. Rep.* **5** 9850
Hei T K, Komatsu K, Hall E J and Zaider M 1988 Oncogenic transformation by charged particles of defined LET *Carcinogenesis* **9** 747–50
Howard M E, Beltran C, Anderson S, Tseung W C, Sarkaria J N and Herman M G 2018 Investigating dependencies of relative biological effectiveness for proton therapy in cancer cells *Int. J. Part. Ther.* **4** 12–22
Jones B 2015 A simpler energy transfer efficiency model to predict relative biological effect for protons and heavier ions *Front. Oncol.* **5**
Mairani A *et al* 2017 A phenomenological relative biological effectiveness approach for proton therapy based on an improved description of the mixed radiation field *Phys. Med. Biol.* **62** 1378
Manganaro L, Russo G, Cirio R, Dalmasso F, Giordanengo S, Monaco V, Muraro S, Sacchi R, Vignati A and Attili A 2017 A Monte Carlo approach to the microdosimetric kinetic model to account for dose rate time structure effects in ion beam therapy with application in treatment planning simulations *Med. Phys.* **44** 1577–89
McNamara A L, Schuemann J and Paganetti H 2015 A phenomenological relative biological effectiveness (RBE) model for proton therapy based on all published *in vitro* cell survival data *Phys. Med. Biol.* **60** 8399
Paganetti H 2014 Relative biological effectiveness (RBE) values for proton beam therapy. Variations as a function of biological endpoint, dose, and linear energy transfer *Phys. Med. Biol.* **59** R419
Patel D *et al* 2017 Optimization of Monte Carlo particle transport parameters and validation of a novel high throughput experimental setup to measure the biological effects of particle beams *Med. Phys.* **44** 6061–73
Peeler C R 2016 *Assessing the Potential Clinical Impact of Variable Biological Effectiveness in Proton Radiotherapy PhD Thesis* (Houston, TX: University of Texas)
Polster L *et al* 2015 Extension of TOPAS for the simulation of proton radiation effects considering molecular and cellular endpoints *Phys. Med. Biol* **60** 5053
Prise K M, Folkard M, Davies S and Michael B D 1990 The irradiation of V79 mammalian cells by protons with energies below 2 MeV. Part II. Measurement of oxygen enhancement ratios and DNA damage *Int. J. Radiat. Biol.* **58** 261–77
RBE Committee 1963 Report of the RBE committee to the international commissions on radiological protection and on radiological units and measurements *Health Phys.* **9** 357–84
Rohatgi A 2018 *WebPlotDigitizer* version 4.2 (Austin, TX) (https://automeris.io/WebPlotDigitizer)
Rørvik E, Fjæra L F, Dahle T J, Dale J E, Engeseth G M, Stokkevåg C H, Thörnqvist S and Ytre-Hauge K S 2018 Exploration and application of phenomenological RBE models for proton therapy *Phys. Med. Biol.* **63** 185013
Schettino G, Folkard M, Prise K M, Vojnovic B, Bowey A G and Michael B D 2001 Low-dose hypersensitivity in Chinese Hamster V79 cells targeted with counted protons using a charged-particle microbeam *Radiat. Res.* **156** 526–34
Schuemann J, Bassler N and Inaniwa T 2018 Computational models and tools *Med. Phys.* **45** e1073–85
Schuff J A *et al* 2002 Relative biological effectiveness measurements of low energy proton and lithium beams on tumor cells *Nucl. Instrum. Methods Phys. Res.* B **187** 345–53
Tommasino F and Durante M 2015 Proton radiobiology *Cancers* **7** 353–81