Original Research Article

Effect of heterogeneous radio sensitivity on the survival, alpha beta ratio and biologic effective dose calculation of irradiated mammalian cell populations

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A B S T R A C T

It is demonstrated that the surviving fraction of a population of cells with heterogeneous radio sensitivity, like that composing most malignant tumors, conforms to a different linear quadratic survival relation for dose less than about 3–5 Gy and dose greater than about 7–9 Gy. In the intermediate range of dose the survival relation for the population, as a whole, is not linear quadratic. Consequently, the value of the alpha beta ratio and the associated biologically effective dose calculation are different for the low and high dose range for most malignant tumors.

Normal tissue cell populations responsible for organ function also have heterogeneous radio sensitivity, though to less degree than most malignant tumors. Consequently, the alpha beta ratio and associated biologically effective dose calculation related to the development of some acute early and chronic late developing radiation injuries are not the same in the low and high dose range.

Variance of the distribution of α of a heterogeneous cell population lowers the effective value of the quadratic survival constant β of the population, as a whole, and increases the α/β ratio in the low dose range. Heterogeneous appearance of tumor cells (pleomorphism) and necrosis on biopsy or imaging studies reflect heterogeneity of the radio sensitivity of the cells. Greater heterogeneity implies a tendency to higher α/β ratio. This may furnish a clinically accessible way to estimate a value of the α/β ratio specific to an individual patient and tumor.

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1. Introduction

The fraction of a homogeneous population of cells, all of which have the same sensitivity to ionizing radiation, that survives instantaneous exposure to dose d Gy is described by the linear quadratic relation,

$$S = e^{-\alpha d - \beta d^2} \quad \text{or} \quad -\ln S = \alpha d + \beta d^2 \qquad (1)$$

An example of linear quadratic survival of a homogenous cell population consisting of V79 cells selected to be in the G1 phase of the cell cycle is shown in Fig. 1 in which the usual plot of $-\ln S$ versus d has been replaced by a plot of $-\ln S/d$ versus d [1]. When plotted as $-\ln S/d$ versus d the linear quadratic dependence of surviving fraction on dose is transformed into linear dependence of the ratio $-\ln S/d$ on d. Both axes have a linear scale making linear

quadratic survival, and deviation from it, recognizable by inspection. This representation of the dose dependence of surviving fraction is used in the figures and discussion here.

In contrast to the linearity of Fig. 1, asynchronous exponentially growing mammalian cell cultures have a more complex survival relation illustrated by that of the HeLa cells [2] shown in Fig. 2. It has been pointed out that curvature in the intermediate dose range and convergence to the lines A and B in the low and high dose limits can be attributed to a mixture of cells in the sensitive and resistant phases of the cell replication cycle that make up the asynchronous irradiated population [3].

The difference between the complex survival versus dose relation that is found for a population that is a mixture of cells of diverse linear quadratic radio sensitivity and that of a population with homogenous radio sensitivity is described and its implications are discussed here. It has been shown that the survival of a heterogeneous cell population instantaneously exposed to ionizing radiation is governed by a linear quadratic relation to dose in the
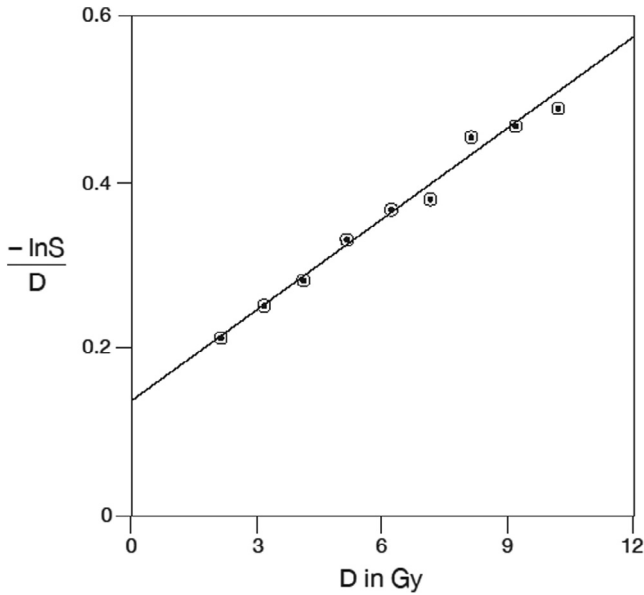
Fig. 1. Surviving fraction of a culture of Chinese hamster V79 cells synchronized in the G1 phase of the cell replication cycle so as to be homogeneous in radio sensitivity. α equals 0.14 Gy^{-1}. β equals 0.037 Gy^{-1} The α/β ratio is 3.8 Gy. Redrawn from Chapman [1].
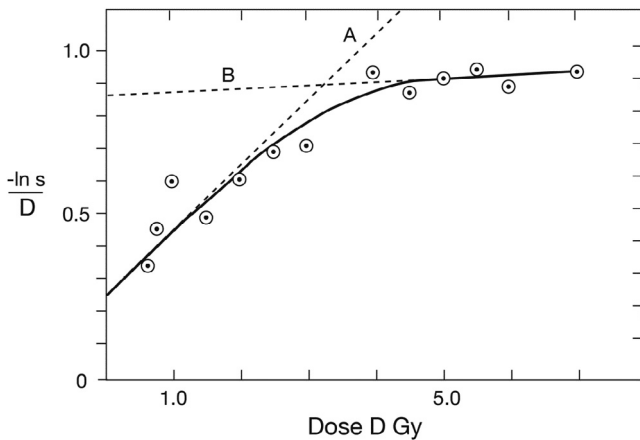

Fig. 2. Surviving fraction of a culture of HeLa cells from Puck and Marcus [2]. From line A α is 0.25 Gy^{-1} and β is 0.19 Gy^{-2}. From line B α_H is 0.84 Gy^{-1} and β_H is 0.013 Gy^{-2}. α/β is 1.13 Gy. α_H/β_H is 64.6 Gy. α_H/α is 3.4, β_H/β is 0.068. The value of d^* is 3.5 Gy. The non linear-quadratic transition range is from about 3 to 4.5 Gy. The lines and the curve were drawn by inspection.

low dose range as dose decreases to approach zero [4]. In part II it is shown that the survival is governed by a different linear quadratic relation in the high dose range, as dose increases toward infinity. The survival relations in the low and high dose linear quadratic ranges are connected by a survival relation for the intermediate transition range of dose that is not linear quadratic. This is illustrated in several of the figures and most clearly in Fig. 2. Part III derives the changes in the calculation of the biologically effective dose (BED) that result from heterogeneous radio sensitivity. Experimental examples and implications of heterogeneous population survival are discussed in parts IV and V

The survival of some cells deviates from the linear quadratic relation as dose decreases to less than about 0.5–1 Gy because of low dose hypersensitivity [3,5]. This effect is not included in the present discussion. The surviving fraction versus dose relation for dose less than about 1.0 Gy is regarded here as that obtained by

extrapolation of the linear quadratic relation established for dose greater than about 1.0 Gy.

2. Survival of a cell population with heterogeneous radio sensitivity

Consider a population of cells with heterogeneous radio sensitivity to consist of M homogeneous subpopulations. Let the i th subpopulation consist of N_i cells with linear quadratic survival constants equal to α_i and β_i. Let N be the number of cells in the combined population, S be the surviving fraction of the cells of the combined population and S_i be the surviving fraction of the i th sub population. The value of S will be the cell number weighted average of the surviving fraction of each of the constituent homogenous sub populations. This is,

$$S = \sum_{i=1}^{M} \frac{N_i}{N} S_i = \sum_{i=1}^{M} \frac{N_i}{N} e^{-\left(\alpha_i d + \beta_i d^2\right)} \tag{2}$$

Let $\langle \alpha_i \rangle$ and $\langle \beta_i \rangle$ be the cell number weighted averages of the survival constants of the various sub populations and $\langle \delta \alpha_i^2 \rangle$ be the variance of the distribution of the values of α_i. It has been shown that [4],

$$-\frac{\ln S}{d} = \langle \alpha_i \rangle + \left(\langle \beta_i \rangle - \frac{1}{2}\langle \delta \alpha_i^2 \rangle\right)d - \frac{1}{4}\langle \delta \alpha_i^2 \rangle^2 d^3 + \ldots \ldots$$

$$\approx \langle \alpha_i \rangle + \left(\langle \beta_i \rangle - \frac{1}{2}\langle \delta \alpha_i^2 \rangle\right)d \tag{3}$$

Thus, as dose decreases to approach zero, so that terms with power of d greater than 1 can be neglected, the value of $-\ln S/d$ as a function of d is approximated by the linear relation of the second line of Eq. (3). This implies linear quadratic dependence of population cell survival for the limiting low dose range. The line represented by the approximate relation in Eq. (3) is referred to below as line A and so labeled in the figures.

Per Eq. (3), the intercept at zero dose of line A is $\langle \alpha_i \rangle$. The slope of line A equals $\langle \beta_i \rangle$ minus one half of $\langle \delta \alpha_i^2 \rangle$. In what follows, when the symbols α and β appear without subscript they will mean the zero dose intercept and slope of line A. That is, α means $\langle \alpha_i \rangle$ and β means $\langle \beta_i \rangle - \langle \delta \alpha_i^2 \rangle / 2$. The values of α and β express the linear quadratic survival of the population as a whole in the low dose limit. For the HeLa cells of Fig. 2, the low dose range in which survival is approximately linear quadratic, is for dose less than about 3 Gy. The upper limit of the low dose linear quadratic range for the malignant tumor and normal tissue cell populations shown in the several figures, and for those in the literature discussed below [6] ranges from about 2–5 Gy.

As shown most clearly in Fig. 2, the $-\ln S/d$ relation becomes non linear as dose exceeds that of the low dose linear quadratic range marked by line A. As dose increases further, $-\ln S/d$ converges to the line labeled B, indicating a second range of linear quadratic survival develops as dose increases toward infinity.

This can be explained and justified as follows. As dose increases the value of each of the terms that make up the summation in Eq. (2) decreases and the sum becomes dominated by the term that decreases least. Let β_H be the lowest value of β_i in the population. Let α_H be the lowest value of α_i to be found among those cells for which β_i equals β_H. The cells with survival constants α_H and β_H define the homogenous sub population that is represented by the term in Eq. (2) that decreases least with increasing dose. In the limit of large dose, the survival of the whole population approaches the linear quadratic survival of this, the most radio resistant homogeneous sub population. This is,

$$\lim_{d\to\infty} S = S_H = \frac{N_H}{N} e^{-(\alpha_H d + \beta_H d^2)} \tag{4}$$

Taking the log of S_H

$$-\ln S_H = -\ln \frac{N_H}{N} + \alpha_H d + \beta_H d^2 \quad \text{or} \quad \frac{-\ln S_H}{d}$$

$$= -\frac{1}{d} \ln \frac{N_H}{N} + \alpha_H + \beta_H d \tag{5}$$

As dose increases to approach infinity this becomes,

$$\frac{-\ln S_H}{d} = \alpha_H + \beta_H d \tag{6}$$

The line labeled B in the figures is Eq. (6). The slope of line B is β_H and its intercept at zero dose is α_H. The values of α_H and β_H must be greater than or equal to zero because they are the linear quadratic survival constants of a homogeneous sub population of cells.

A horizontal line on a plot of $-\ln S/d$ as a function of d corresponds to β_H equal to zero and purely exponential survival. The exponential survival that is found in the high dose range in many survival experiments with cultured mammalian cells is explained by heterogeneity of radio sensitivity of an irradiated cell population that has a component with zero or near zero value of β_H.

Let d^* be the dose at which the limiting low and high dose lines intersect. The value of d^* is determined by solving Eqs. (3) and (6) as simultaneous equations for d. This gives,

$$d^* = \frac{\alpha_H - \alpha}{\beta - \beta_H} = \frac{\alpha_H - \langle \alpha \rangle}{\langle \beta \rangle - \frac{1}{2}\langle \delta\alpha^2 \rangle - \beta_H} \tag{7}$$

Note that d^* depends only on the difference between α and α_H and between β and β_H. If β equals β_H and α equals α_H, lines A and B merge into a single line to represent the linear quadratic survival of a homogeneous population. The population cell survival is approximated by the linear quadratic relation with constants α and β for dose less than the transition range and with constants α_H and β_H for dose greater than the transition range.

3. Biologically effective dose of a fractionated course of radiation treatment

Consider a course of F exposures of a population of dividing cells to repeated instantaneously administered dose of d Gy. Each exposure is referred to as a fraction and follows the preceding fraction by time sufficient to allow for completion of repair. The biologically effective dose ($BED_{\alpha/\beta}$) of such a course is defined so as to be the total dose that would have to be given to produce the same surviving fraction as that produced by the course of interest, if the fractional dose were made to approach zero so that the contribution of the quadratic survival term becomes effectively zero [6]. The defining relation for $BED_{\alpha/\beta}$ is,

$$-\ln S_F = \alpha BED_{\alpha/\beta} \tag{8}$$

In which S_F is the number of viable cells present immediately after the last exposure divided by the number of viable cells present immediately prior to the first. The value of α is the linear survival constant that governs survival of the irradiated population in the limit of low dose. If the effect of radiation of different linear energy transfer (LET) is being compared, the value of α in Eq. (8) is that of the reference low LET radiation, usually high energy xrays.

For a homogeneous population of cells, all of which have the same value of the survival constants α and β, Eqs. (1) and (8) imply [6–8],

$$BED_{\alpha/\beta} = \frac{-\ln S_F}{\alpha} = \frac{-F\ln s - \frac{T\ln 2}{\tau_2}}{\alpha} = \frac{F(\alpha d + \beta d^2)}{\alpha} - \frac{T\ln 2}{\alpha \tau_2}$$

$$= D\left(1 + \frac{\beta}{\alpha} d\right) - \frac{0.693T}{\alpha \tau_2} \tag{9}$$

In which s is the fraction of the cells that survive each exposure to dose d. The value of D equals $F \times d$. The value of T is the time between the first and last exposures and τ_2 is the volume doubling time of the cell population as measured in the absence of radiation exposure. The time dependent term accounts for the increase in viable cells from cell replication during the course [7,8].

For a heterogeneous cell population, for d less than the transition range of dose so that survival is approximated by line A, the value of $BED_{\alpha/\beta}$ is given by Eq. (9) with the understanding that, per the second line of Eq. (3), α equals $\langle \alpha_i \rangle$ and β equals $\langle \beta_i \rangle - \langle \delta\alpha_i^2 \rangle / 2$.

Let $BED_{\alpha_H/\beta_H}^H(d)$ be the biologically effective dose calculated for dose d great enough that $-\ln S_F/d$ is approximated by the high dose linear asymptotic relation of Eq. (6) (line B). Then,

$$BED_{\alpha_H/\beta_H}^H(d) = \frac{-F\ln S_F}{\langle\alpha\rangle} = \frac{F(\alpha_H d + \beta_H d^2)}{\alpha} - \frac{0.693T}{\alpha \tau_2}$$

$$= \frac{\alpha_H}{\alpha}\left[D\left(1 + \frac{\beta_H}{\alpha_H} d\right) - \frac{0.693T}{\alpha_H \tau_2}\right] = \frac{\alpha_H}{\alpha} BED_{\alpha_H/\beta_H}(d) \tag{10}$$

Note that the symbol BED_{α_H/β_H} refers to the biologically effective dose of Eq. (9) with α_H and β_H in place of α and β.

The biologically effective dose appropriate to a course of radiation treatment with fractional dose d less than that of the transition dose range (line A) is $BED_{\alpha/\beta}$ calculated with Eq. (9). That appropriate to a course with d greater than the transition dose range (line B) is BED_{α_H/β_H}^H calculated with Eq. (10). The value of BED for dose in the transition range will be between $BED_{\alpha/\beta}$ and BED_{α_H/β_H}^H and can be estimated by interpolation. Calculated this way, it is valid to compare the BED of a course with d less than the transition range with one for which d is within or greater than the transition dose range. The value of the ratios α/β, α_H/β_H, α_H/α and β_H/β can be obtained from an iso effect study (see appendix).

The Eqs. (9) and (10) expressions for biologically effective dose implicitly include the assumption that $\langle \alpha_i \rangle$, $\langle \beta_i \rangle$, $\langle \delta\alpha_i^2 \rangle$ and the volume doubling time of the population in the absence of irradiation, do not change during a fractionated course. However, the distribution of the values of α and β that determine $\langle \alpha_i \rangle$, $\langle \beta_i \rangle$, $\langle \delta\alpha_i^2 \rangle$ may vary during a course of treatment due to recruitment of quiescent cells into the division cycle and redistribution of cells within the division cycle. The distributions may also vary with changes in cellular environment such as reoxygenation. The rate of growth of the cell population may decrease relative to that of the unirradiated population because of radiation induced delay in the progress of cells through the division cycle. The growth rate may increase because of stimulation from chemical signals that are produced in response to radiation exposure. Growth rate increase from irradiation is termed accelerated repopulation [9]. To account for accelerated repopulation a lag time is sometimes subtracted from the value of T in Eqs. (9) and (10).

4. Discussion: Malignant tumors

Heterogeneity of radio sensitivity occurs in malignant tumors from the presence of quiescent cells, cells at various stages in the division cycle and from variation of cellular environment due to limited access to the circulation, particularly limited access which produces zones of hypoxia. There also may be genetically distinct clones in some malignant tumors.
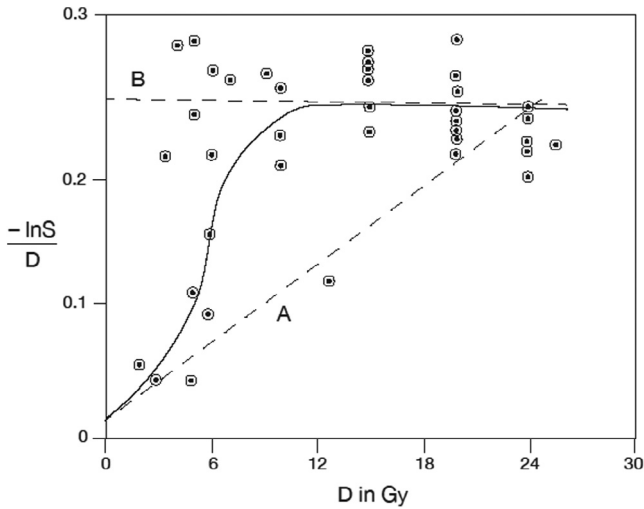
Fig. 3. Surviving fraction of cells of sc9L rat gliosarcoma tumors irradiated in air breathing mice. From Moulder and Rockwell [10]. From line A α equals 0.015 Gy^{-1} and β equals 0.01 Gy^{-2}. The value of α/β is 1.5 Gy. From line B α_H is 0.26 Gy^{-1} and β_H is zero. The ratio α_H/β_H is infinite. The ratio α_H/α is 17.3. The ratio β_H/β is zero. The non linear quadratic transition range is from about 2 to 10 Gy. The lines and curve were drawn by inspection.
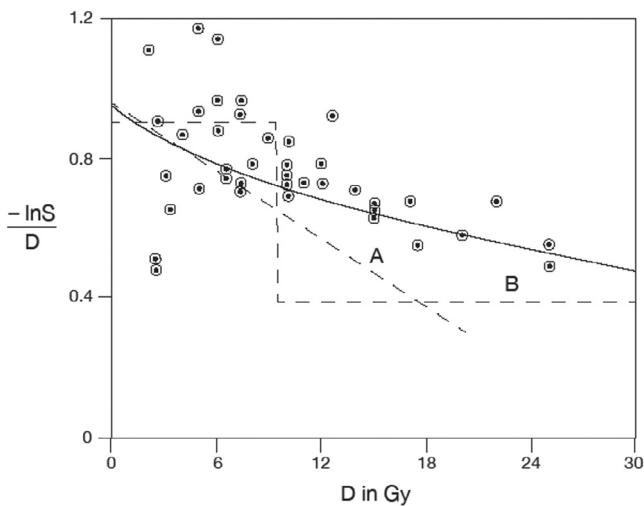

Fig. 4. Surviving fraction of cells of a subcutaneous solid lymphosarcoma irradiated in air breathing mice replotted from Powers and Tolmach [11]. From line A α equals 0.96 Gy^{-1} and β equals negative 0.032 Gy^{-2}. The value of α/β is negative 30 Gy. From line B α_H is 0.5 Gy^{-1} and β_H is zero. The ratio α_H/β_H is infinite. The ratio α_H/α is 0.52. β_H/β is zero. The value of d^* is 17.5 Gy. The non linear-quadratic transition range is from about 5 to 30 Gy. The lines and the curve were drawn by inspection. The horizontal and vertical dashed line segments correspond to the two intersecting exponential survival relations drawn in Ref. [11].

Examples of the survival of cells of experimental mouse tumors with a significant hypoxic component are shown in Figs. 3 and 4. There is considerable random variation of surviving fraction measurements made on tumor cell populations irradiated in situ in an animal. This is amplified in the ratio $-\ln S/d$ for low values of d. The curves drawn in Figs. 3 and 4 to represent the $-\ln S/d$ versus d relation, in addition to being determined by the experimental values shown in the figures, were also in part determined by constraints indicated in Eqs. (3) and (6). The constraints are that there be linear dependence in the low and high dose limits with zero or positive values of $\langle \alpha_i \rangle$, α_H and β_H.

As illustrated in Figs. 2 and 3, if $\beta > \beta_H$, the value of α_H will be greater than α, and $\alpha/\beta < \alpha_H/\beta_H$. Since β_H is the lowest value of the set of values of β_i that make up $\langle \beta_i \rangle$, it is expected that β will

usually be greater than β_H. However, per Eq. (3), the value of β is decreased from that of $\langle \beta_i \rangle$ by variance of the distribution of α_i so it is possible for β_H to be greater than β. It is even possible for β to be negative if $\langle \beta_i \rangle$ is less than one half of the variance of α_i. This occurs if there are large differences in the values of α_i or most of the values of β_i are zero.

An example of a negative value of β, is shown in Fig. 4 which shows the survival of a mouse lymphosarcoma tumor [11]. The experimental values plotted as $-\ln S$ versus d in Ref. [11] indicate two sub populations, each with apparently pure exponential survival, one aerated and the other hypoxic. The combination of the two purely exponential survival components as drawn in Ref. [11] is transformed to the horizontal dashed line segments connected by a vertical dashed line segment shown in Fig. 4. The survival relation drawn as the solid curve in Fig. 4 has an initial slope that is negative corresponding to a negative β. The experimental values are consistent with negative β but their scatter is such that they don't indicate any particular value for the initial slope. A negative initial slope is suggested by the negative slope defined by the points at higher dose. Per Eq. (6), the $-\ln S/d$ versus d relation must converge to a line that has zero or positive slope as dose increases toward infinity. It is drawn as approaching a line with zero slope in Fig. 4.

Experiments with cultured mammalian cells [2,3] like that shown in Fig. 2 indicate the range of transition between line A and B survival is from about 3 to 5 Gy. For the malignant animal tumor [10,11] shown in Fig. 3 the transition range is from about 3 to 15 Gy. For that of Fig. 4 the transition range extends from about 5 to as high as about 25 Gy or more. From Figs. 2, 3 and 4 the values of the α/β ratio are, respectively, 1.13, 1.5, and negative 29 Gy. The values of the α_H/β_H ratio are, respectively, 64.6, infinity and infinity.

The consequence of a negative α/β ratio is that, in the low dose range, increasing the fractional dose while maintaining the total dose of the course constant, decreases $BED_{\alpha/\beta}$ instead of increasing it. The consequence of a very low or zero value of β_H is that in the high dose linear quadratic range the ratio α_H/β_H is effectively infinite and the β_H/α_H ratio is effectively zero. This causes BED_{α_H/β_H}^H to be unaffected by change in fractional dose within the high dose range if total dose is maintained constant.

Values of the alpha/beta ratio of experimental and human tumors are tabulated in several places [12–15]. Most of the values lie between about 5 and 15 Gy but with unusual values as low as 1 Gy and as high as 50 Gy. The span of fractional dose used to obtain the tabulated alpha beta ratios, when recorded [12,14], is about 6–20 Gy. Alpha/Beta ratios obtained from this dose range are likely greater than α/β and closer to, but not as great as, α_H/β_H.

The values of α/β, α_H/β_H and the limits of the non linear transition dose range for a malignant tumor targeted for radiation treatment will in general not be known. As the tabulated values indicate, the range of possible values of the alpha beta ratio is very large. Absent a better estimate of α/β for the calculation of $BED_{\alpha/\beta}$, a default value of 10 Gy is often selected. This is in the middle of the range of most of the tabulated alpha beta ratio values for malignant tumors.

Consider that the value of β will usually be greater than that of β_H, so that α/β will be less than α_H/β_H. Further, the tabulated values of the alpha beta ratio are, for the most part, estimated from a range of dose that is mostly greater than the middle of the transition dose range so that the estimated values of the alpha beta ratio in the tables are likely nearer to α_H/β_H than to α/β. This suggests a default choice of α/β from the low end of the reported range, for instance 3–5 Gy, may be better for calculating $BED_{\alpha/\beta}$ for tumor control by a course of radiation with fractional dose less than about 5 or 6 Gy. For a heterogeneous malignant tumor the value of β_H will

often be near or equal to zero. The calculation of $BED^H_{\alpha_H/\beta_H}$ for fractional dose greater than about 10–15 Gy can usually be with α_H/β_H equal to infinity.

From Eq. (3), form an expression for the reciprocal of the α/β ratio.

$$\frac{\beta}{\alpha} = \frac{\langle\beta_i\rangle - \frac{1}{2}\langle\delta\alpha_i^2\rangle}{\langle\alpha_i\rangle} = \frac{\langle\beta_i\rangle}{\langle\alpha_i\rangle} - \frac{1}{2}\frac{\langle\delta\alpha_i^2\rangle}{\langle\alpha_i\rangle} \qquad (11)$$

This implies diversity in the value of α_i, expressed in its relative variance, will tend to decrease the population's β/α ratio and increase the α/β ratio.

Eq. (11) suggests a clinically accessible basis with which to estimate the value of the α/β ratio specific to an individual patient and tumor. Consider that heterogeneity of appearance of cells on an H and E stained microscope slide reflects heterogeneity of DNA content and cell access to capillary circulation. These features are related to cellular radio sensitivity. This suggests that the degree of variation in the appearance of the nuclei and cells of a tumor on a biopsy slide, referred to as pleomorphism, may correlate with heterogeneity of radio sensitivity. The presence of necrosis in the biopsy, or on imaging studies, is an indication of an hypoxic component and consequently also of increased heterogeneity. The more heterogeneous the radio sensitivity of a tumor cell population, the greater the value of $\langle\delta\alpha_i^2\rangle$ and, per Eq. (11), the lower the value of β/α and the higher the value of α/β. For instance, a value of the α/β ratio in the range of 10–15 Gy or higher may be appropriate for a tumor that shows necrosis on imaging studies or a relatively high degree of pleomorphism on its biopsy slides. A value in the range of 3–5 Gy, or even lower as is found for prostate carcinoma [16], may be more appropriate if there is no indication of necrosis and the biopsy shows a uniform appearing population of cells with little or no pleomorphism.

5. Discussion: Normal tissues

Acute early and chronic late developing radiation injury is caused by the death of cells of the parenchyma and stroma of an irradiated organ. Like malignant tumor cell populations, the cell population of an organ may include cells that are not cycling and others at various stages in the cell replication cycle. Unlike that of a malignant tumor, the capillary bed of normal tissues is organized to provide the constituent cells with ample uniform access to the circulation and the cell population of normal tissues does not contain genetically distinct clones. As a result, the cell populations upon which the function and integrity of normal organs depend tend to have more nearly homogeneous radio sensitivity than those of malignant tumors. This is particularly true of those related to chronic late developing radiation injury that are mostly quiescent during a fractionated course of radiation therapy.

Fig. 5 shows the survival of mouse jejunal crypt cells [18] as an example of survival of a normal tissue cell population related to acute early developing radiation injury. The experimental values of $-\ln S/d$ are shown with lines A and B drawn as for a heterogeneous population. The heterogeneity is likely that of a mixture of cells at various phases of the cell division cycle.

Fig. 6 shows the dependence of $1/D^\otimes$ on the fractional dose that produces hind leg paralysis in 50 percent of exposed mice [19]. This is an example of an isoeffect study of a chronic late developing radiation injury, as described in the appendix. The cells of the spinal cord that determine the late development of paralysis appear to have relatively homogeneous radio sensitivity with α_H/α equal to 2.0 and β_H/β equal to 0.57. Nevertheless, the difference between α/β equal to 2.6 and α_H/β_H equal to 8.3 makes a significant difference in the calculation of the BED for spinal cord injury for irradiation with fractional dose less than or greater than 3–5 Gy. The cells relevant to the spinal cord paralysis are thought to be mostly in a quiescent state during the course of radiation treatment. What heterogeneity of radio sensitivity that is present may be from the end point of paralysis being determined by the survival of a population of cells made up of two or more sub populations of different histology, for instance, astrocytes, oligodendrocytes and vascular endothelium.

Values of the alpha beta ratio that characterize the radiation response of normal tissues have been tabulated [13–15]. They tend to be in the range of 5–15 Gy for acute early developing radiation injury, examples of which include moist desquamation, pharyngitis, esophagitis, diarrhea and dysuria. For chronic late developing radiation injury tabulated values of the alpha beta ratio tend to be in the range of 1–7 Gy [13–15]. Examples of late radiation injury include spinal paralysis, brain necrosis, pulmonary fibrosis, bowel stricture and kidney failure. Heterogeneity from variation of radio sensitivity among phases of the cell replication cycle is thought to be more pronounced in the cells related to the early injury because
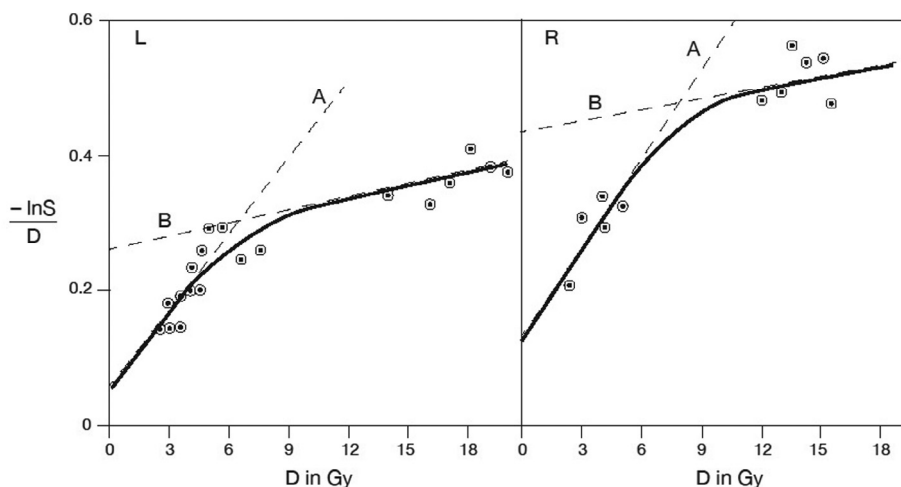

Fig. 5. Surviving fraction of jejunal crypt cells from mice exposed to a single dose of gamma rays. Panel L is for mice given radioprotectant WR2721 and Panel R is without WR2721. Panel L: α equals 0.06 Gy^{-1}, β equals 0.04 Gy^{-2}, the ratio α/β is 1.5 Gy; α_H is 0.27 Gy^{-1}, β_H is 0.007 Gy^{-2}, the ratio α_H/β_H is 38.6 Gy, the ratio α_H/α is 4.5, the ratio β_H/β is 0.18. d^* is 6 Gy. The non linear quadratic transition range is from about 4.5 to 9 Gy. Panel R: α equals 0.12 Gy^{-1}, β equals 0.045 Gy^{-2}, the ratio α/β is 2.7 Gy, α_H is 0.43 Gy^{-1}, β_H is 0.004 Gy^{-2}, the ratio α_H/β_H is 107.5 Gy, the ratio α_H/α is 3.6, the ratio β_H/β is 0.09. d^* is 7.5 Gy. The non linear quadratic transition range is from about 5.5 to 12 Gy. Redrawn from Travis et al. [18].
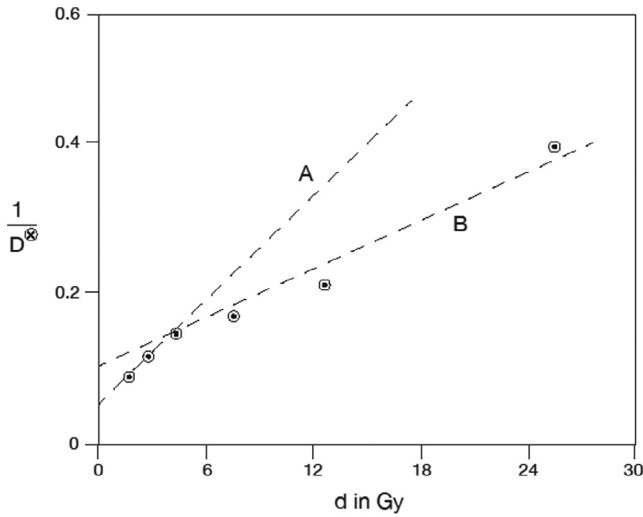
Fig. 6. $D^{\otimes}$ is the dose that causes lumbar spinal cord myelopathy in mice exposed to a fractionated course of radiation of fractional dose d. From line A the value of α/β is 2.36 Gy. From line B the ratio α_H/β_H is 8.3 Gy. The ratio α_H/α is 2.0. β_H/β is 0.57. The value of d^* is 4 Gy. The non linear quadratic transition range is from about 4 to 5 Gy. The lines were drawn by inspection. From Hornsey and White [19].

of their active replication during the radiation course. The cells related to chronic late developing injury are thought to be mostly quiescent during a course of radiation treatment and as a result more homogeneous in radio sensitivity. Eq. (11) suggests that the tendency to a higher alpha beta ratio for early relative to that of late developing radiation injury may be due, at least in part, to greater heterogeneity expressed in a greater value of the $\langle \delta\alpha_i^2 \rangle / \langle \alpha_i \rangle$ ratio. This explanation of the higher α/β ratio of early radiation injury has been suggested based on the assumption of a bivariate normal distribution of the α_i and β_i values of replicating cells [17].

Barendsen [6] has assembled iso effect relations for an array of normal tissue injuries produced by fractional irradiation. These are presented in the form of $1/D^{\otimes}$ versus d as described in the appendix and sketched in Fig. 7. Acute early developing injuries include for $D^{\otimes}$ equal to the dose that produces 10 per cent survival of mouse jejunal crypt cells [20]; $D^{\otimes}$ equal to the dose that produces 50 per cent death of mice at 30 days from bone marrow failure [21]; and $D^{\otimes}$ equal to the dose that produces moist desquamation of mouse foot pad [22]. The experimental values of $1/D^{\otimes}$ versus d
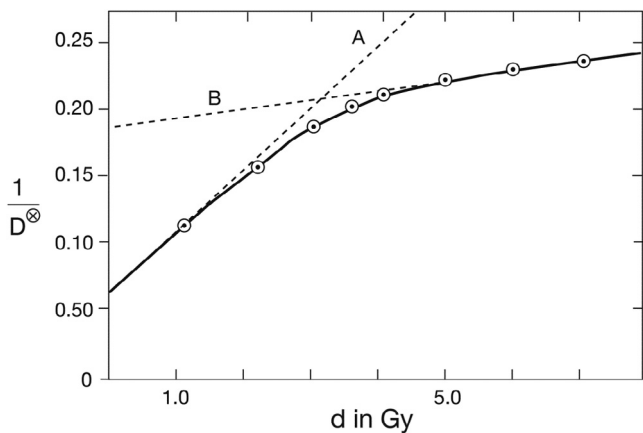

Fig. 7. Schematic of relation between the reciprocal of the total dose of a fractionated course of radiation needed to produce a given effect versus the fractional dose (isoeffect plot). Line A represents linear quadratic survival of the cell population as a whole that is approached as fractional dose approaches zero and line B is the line representing linear quadratic survival approached as fractional dose becomes large.

for each of these isoeffects can be fit to a single straight line consistent with homogeneous radio sensitivity of the associated cell population. However, with the exception of the moist desquamation of skin, there is a suggestion of downward concavity of a transition range of dose in each so that a pair of low and high dose lines like A and B in Fig. 7, with hinge angle near, but not equal to 180 degrees, provides a better fit. The transition dose range for the early developing injuries as shown in Fig. 5 and discussed by Barendsen [6] is from about 3 to 9 Gy.

Chronic late developing injuries considered by Barendsen [6] include those for $D^{\otimes}$ equal to the dose that produces 50 percent death of mice by 180 days from respiratory failure [3,23,24]; $D^{\otimes}$ equal to the dose causing 50 percent death by 180 days from kidney failure [25]; $D^{\otimes}$ equal to the dose that produces 30 percent contraction of mouse skin by 250 days [26]; $D^{\otimes}$ equal to the dose that produces paralysis of the hind legs of 50 percent of rats from radiation myelopathy of the spinal cord [27–29]; and 50 percent death of rats by one year from brain irradiation [30]. Like that for the acute early injuries there is a suggestion of downward concavity of the iso effect relation. The experimental values of $1/D^{\otimes}$ versus d for each of these isoeffects can be fit to a single straight line consistent with homogeneous radio sensitivity or, with better fit to a pair of lines like A and B of Fig. 7. The transition dose range for the late developing injury shown in Fig. 6 and discussed in Barendsen [6] is from about 3 to 6 Gy.

Conflict of interest statement

The author declares no conflict of interest.

Appendix: Isoeffect studies

Let $D^{\otimes}$ be the total dose of a course of radiation treatment consisting of F exposures of d Gy each over time span T, that produces an effect such as a specific level of organ failure or tumor control. For instance, $D^{\otimes}$ may be the dose that controls an irradiated tumor 50% of the time, or that produces moist desquamation of skin or paralysis of the lower extremities of 50% of the exposed animals. It is assumed that the specified effect develops when the viable fraction of the population of cells responsible for maintaining the organ function or tumor viability is decreased to below a critical level by exposure to ionizing radiation. Let this critical survival fraction of the responsible population be $S^{\otimes}$. The value of $S^{\otimes}$ will generally not be known. However, it will be the same no matter what combination of fractional and total dose constitutes the course of irradiation treatment that produces the effect. An effect of radiation studied this way is referred to as an isoeffect and $D^{\otimes}$ is the iso effect dose [6,7].

Let the responsible cell population have homogeneous radio sensitivity so that all its cells have the same linear quadratic survival constants α and β. Let the critical survival level be reached after F fractional exposures of dose d Gy over a time period of duration T. Then,

$$-\ln S^{\otimes} = F \ln s - \frac{0.693T}{\tau_2}$$

$$= F(\alpha d + \beta d^2) - \frac{0.693T}{\tau_2} \quad \text{with} - \ln s = \alpha d + \beta d^2$$

$$= D^{\otimes}(\alpha + \beta d) - \frac{0.693T}{\tau_2} \tag{A1}$$

In which s is the fraction of the cells of the responsible homogeneous population that survive each dose of d Gy. The value of T is the time between first and last exposures and τ_2 is the volume doubling time of the population in the absence of irradiation.

Eq. (A1) can be rewritten as [6,7],

$$\frac{1}{D^{\otimes}} = \left(\frac{\tau_2}{-\tau_2 \ln S^{\otimes} + T \ln 2}\right)\alpha + \left(\frac{\tau_2}{-\tau_2 \ln S^{\otimes} + T \ln 2}\right)\beta d \qquad (A2)$$

The value of T will depend on d unless the time between fractions is adjusted so as to hold T constant. This is not usually done for clinical treatment courses from which the isodose relation for a radiation injury is derived. However, what dependence there is of T on d will not significantly affect the value of the bracketed quantity in Eq. (A2) if the value of $-\tau_2 \ln S^{\otimes}$ is much greater than that of $T \ln 2$. This is likely the case for the late developing injuries that are dose limiting. Assuming one or both these conditions are met, the value of the bracketed quantity in Eq. (A2) will be effectively independent of the value of d so that $1/D^{\otimes}$ will depend linearly on d for a homogeneous cell population with linear quadratic dependence on dose. A plot of $1/D^{\otimes}$ versus d has been referred to as an F_e plot [6,7].

For a cell population with heterogeneous radio sensitivity, the value of $1/D^{\otimes}$ will be linearly related to fractional dose d for those values of d for which the population survival is linear quadratic. These are the limiting low and high dose ranges for which survival is approximated by lines A and B. In the intermediate transition range of dose $1/D^{\otimes}$ will be non linear, usually with concavity downward. An example of this relation is sketched in Fig. 7. An experimental or clinical population of cells with more or less homogeneous linear quadratic radio sensitivity will produce an isoeffect relation for which lines A and B merge into a single line as the hinge angle between them becomes 180 degrees.

For a heterogeneous population of cells Eq. (A2) is that of line A with the understanding that $\alpha = \langle \alpha_i \rangle$ and $\beta = \langle \beta_i \rangle - \langle \delta \alpha_i^2 \rangle / 2$. The equation of line B in Fig. 7 is,

$$\frac{1}{D^{\otimes}} = \left(\frac{\tau_2}{-\tau_2 \ln S^{\otimes} + T \ln 2}\right)\alpha_H + \left(\frac{\tau_2}{-\tau_2 \ln S^{\otimes} + T \ln 2}\right)\beta_H d \qquad (A3)$$

The value of the α/β ratio is the zero dose intercept of line A divided by its slope. The value of the α_H/β_H ratio is the zero dose intercept of line B divided by its slope. If the span of dose includes d^* the value obtained will lie between α/β and α_H/β_H. If the intermediate segment is concave downward, $\alpha_H/\beta_H > \alpha/\beta$. If it is concave upward $\alpha_H/\beta_H < \alpha/\beta$.

The value of the α_H/α ratio is the zero dose intercept of line B divided by that of line A. The value of the β_H/β ratio is the slope of line B divided by that of line A. The values of the α_H/α and β_H/β ratios quantitatively express the degree of heterogeneity of the radio sensitivity of the population by their variation from one.

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