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Robert Katz
University of Nebraska-Lincoln, rkatz2@unl.edu

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High LET Constraints on Low LET Survival

Robert Katz

University of Nebraska–Lincoln, Lincoln, NE 68588, U.S.A.

Abstract

Survival curves for biological cells irradiated with gamma rays (and other low LET radiations) cannot be properly interpreted without examining the implications of these interpretations for high LET radiations. The theory of RBE demands that the RBE of any high LET radiation field is at most one when the probability for cell killing as a function of the absorbed dose of gamma rays is linear or sublinear, for homogeneous samples whose radiosensitivity parameters are not altered during the irradiation. A contrary experimental finding strongly suggests that the experimental sample is heterogeneous. Violation of this constraint is not unusual where the survival curve found with low LET radiations has a well defined initial negative slope.

1. Introduction

Though survival curves for biological cells exposed to radiations of different quality have long been studied, many inconsistencies and disagreements remain. In part this is because models are not predictive, or if they are they do not provide a uniformly good fit to experimental data. Often a model is supported by a detailed narrative which allows a little too much room for adjustment for the comfort of the reader. In part, problems arise because the experimental data are uncertain. One cannot be sure that two experiments are describing exactly the same biological system, or that either of the two makes use of a truly homogeneous sample.

There is thus a need for some relatively straightforward logical constraints upon both experimental data and models, useful in the sense that some presently existing experimental data and models fall short of the constraints and can therefore be rejected.

One such constraint is proposed here:

> *The probability of cell killing relative to absorbed dose, after gamma irradiation, must be supralinear if an RBE greater than 1 is to be observed for any high LET radiation.*

To violate this constraint a model must explicitly include temporal differences between low and high LET radiations.

An experimental violation of the constraint suggests strongly that the cell samples studied are heterogeneous, that their characteristic radiosensitivity parameters are altered during the irradiations, or perhaps that there are dosimetric problems.

2. A logical exercise

In order to examine the probable differences in biological response to low and high LET radiation fields, we must compare the elementary units of the two radi-

ation fields. Both are made up of excitations and ionizations. There are differences in the spatial and in the temporal distributions of these energy transfer events.

Let us restrict our discussion to systems which cannot perceive time intervals shorter than their own characteristic times. If we take this time to be of the order of hours, a radiation dose has the same effect whether deposited randomly in minutes, as with gamma rays, or whether it comes in 10^{-15} s bursts of delta rays, with the bursts themselves spread over minutes, as with beams of heavy ions. Differences in response between the two radiation fields must then arise from differences in the spatial distributions of ionizations and excitations, from the primary and the secondary interactions.

An estimate of the importance of excitations relative to ionizations in producing cellular inactivation may be formed from the quantum yield for inactivation by ultraviolet light. According to Jagger (1967), order of magnitude estimates for the quantum yield for cell killing from ultraviolet light range from 10^{-6} to 10^{-12}, decreasing with increasing size and complexity of the biological cell. We may ignore both primary and secondary excitations relative to ionizations in any discussion of cell killing from ionizing radiations. Thus our discussion must be focused on the spatial distribution of ionizations.

Since most ionizations come from secondary processes, whatever the primary radiation, we need only consider the spatial distribution of ionizations produced by secondary electrons. With heavy ions, for example, delta rays alone deposit sufficient "local dose" near the ion's path to "saturate" detector response in this region.

Each individual sensitive element of the detector knows only that it has been penetrated by one or more electrons, of different velocities, at different times. It does not know their origin. As electrons interact with matter they manufacture secondaries, tertiaries, and higher generation electrons, usually described as the "electron slowing down spectrum." Any sensitive element within a biological detector is then irradiated with a swarm of electrons having different velocities, and since the ionization cross-sections of the atoms and molecules in tissue peak near 100 eV, it is the low energy end of the slowing down spectrum, say below 1 keV, which is instrumental in producing further (and adjacent) ionizations. A detailed Monte Carlo calculation of the slowing down spectrum shows that it is essentially independent of the energy of the primary electrons, from 1 keV to 1 MeV, at secondary electron energies two decades below the primary energy (Hamm, Wright, Katz, Turner, and Ritchie 1977) in liquid water. At the same deposited energy, the number of ionizations and the number of excitations of different sorts are also independent of the primary electron energy. The number of low energy electrons born is the same. Thus the density of individual ionizations, and ionizations correlated because they are from the same low energy electron are all the same, at the same dose level, whether from 10 keV or 1 MeV primary electrons.

At the same local dose level we must expect the same distribution between ionizations and excitations, between isolated and clustered ionizations, without regard to whether the electrons in the slowing down spectrum come from delta rays, from gamma rays, or from energetic electrons. There can be some differ-

ences where the bulk of the primary electrons have energies below 100 eV, as might be found within 100 Å of the path of a heavy primary particle. But such distances involve only a small fraction of the cross-sectional area of a sensitive cellular target, and must be considered as of negligible importance in the overall problem of cell killing by high LET radiations, where inactivation cross-sections are about half a square micron.

Whether from gamma rays or from heavy ions, from low LET radiations or high, the event spectrum within paired subvolumes at the same level of local dose is the same. The difference between low and high LET radiations is in the concentration of ionizing events around the ion's paths in the high LET fields, as compared to the nearly randomly dispersed ionizations through the macroscopic volume in the low LET fields.

If there is an advantage in cell killing from the concentration of ionizing events in high LET radiations, it must be foretold in the dose-response curve for gamma rays.

Let us consider that we have before us a log-log plot of the probability of cell killing by gamma rays against the macroscope dose. Let us further assume that the ionizing events from the radiation field are sufficiently dispersed that the biological cells cannot distinguish any deviation from perfect randomness; that is, the ionizations are far apart compared to cell sensitive elements. We assign to this field a relative effectiveness of 1.

Now consider that the plot before us is a straight line at 45° to the dose axis. For such a detector, whose response to γ-rays is represented by this curve, there is no advantage to be found from concentrating the ionizations into a smaller subvolume, at the same macroscopic dose level as before. Clustering the ionizing events can kill no more cells. Any increase in local dose in some of the subvolumes of the medium increases the probability of cell killing, but for a proportionately smaller population. The total number of cells killed remains the same as before. For such a dose-effect relationship the RBE must be exactly 1, for all high LET radiation fields.

If the plot before us lies beneath the 45° line drawn through the lowest dose point, such a curve is sublinear. Now a concentration of ionizing events makes inefficient use of the ionizations. All dose-effect relations are sublinear in the region of saturation. Some are sublinear everywhere. The more pronounced the sublinearity, the lower the dose at which saturation occurs, the lower the RBE for any particular high LET field. Similarly, the higher the value of z^2/β^2, or the LET, of the particles making up the field, the lower will be the RBE exhibited by the radiation field.

Finally, let us consider the only case in which the RBE can be greater than 1 for any high LET radiation field: the log-log plot of the probability of cell killing relative to absorbed dose lies above a 45° line through its lowest dose point and is thus supralinear. Here there is an advantage to be gained by concentrating the dose in small subvolumes. Only from a dose-response curve after gamma irradiation which is supralinear can the RBE be greater than 1 from a radiation field which concentrates some of the ionizations into small subvolumes, at the same level of macroscopic dose.

When the radiation field is made up of heavy ions, we do not have control of the manner in which ionizing events are concentrated into small subvolumes. The events are distributed radially about each ion's path, with an ionization density which falls approximately as the square of the radial distance from the ion's path. In order to assess the RBE of the high LET field, we must examine the fraction of the subvolumes, of the cylindrical shells whose axis is the ion's path, in which the local dose is in the supralinear region. If a dose-effect relation for gamma rays is part linear, part supralinear, and part sublinear, the volume of these shells in the supralinear part of the gamma ray dose effect relation must more than compensate for the sublinear part if the RBE is to exceed 1. Thus it is a necessary condition that the dose-effect relation for gamma rays be supralinear if the RBE is to exceed 1 with any high LET field. It is not a sufficient condition.

3. Models

The constraint upon supralinearity is reflected in cell survival models as a constraint upon the parameters of different models which are permitted their dose-effect relation for gamma rays, if the model is to describe a biological system for which an RBE greater than 1 is observed with high LET radiations. For the multitarget model, where cell killing is described by the equation

$$K = 1 - N/N_0 = [1 - \exp(-D/D_m)]^m \tag{1}$$

and the multihit model, where cell killing is described by

$$K = \sum_{x=c}^{\infty} (D/D_c)^x \exp(-D/D_c)/x! \tag{2}$$

where m is the number of targets in which at least 1 hit per target is required for killing, c is the number of hits required in a single target, D is the absorbed dose, D_m and D_c are the dose levels at which there is an average of 1 hit per target, the curves are inherently supralinear up to saturation levels so long as m or c is greater than 1. Thus for m or c greater than 1, there is no logical conflict between the mathematical model and the observation of an RBE greater than 1 with high LET radiations.

This is not the case for either the $\alpha-\beta$ model or the 2-component model. Here we must impose a restraint upon the model parameters.

For the $\alpha-\beta$ model the probability for cell killing is given as

$$K = 1 - \exp[-(\alpha D + \beta D^2)]. \tag{3}$$

Since at low doses the killing is a linear function of D

$$\lim_{D\to 0} K = \alpha D \tag{4}$$

we can define a "supralinearity index," S, to be

$$S = K/\alpha D = \{1 - \exp[-(\alpha D + \beta D^2)]\}/\alpha D \tag{5}$$

whose value is less than, equal to, or greater than 1 when the probability for cell killing is sublinear, linear, or supralinear. We find the "threshold" of supralinear-

ity to be given by $\alpha^2/\beta = 1.5$. When this quotient is *less* than 1.5, the $\alpha-\beta$ model begins to display supralinearity. When $\alpha^2/\beta > 1.5$, the model is linear or sublinear. If the probability for cell killing after exposure to gamma rays is fitted by this model and α^2/β is above the supralinearity threshold, and the cellular system exhibits an RBE greater than 1 for any high LET radiation field, we must question the meaning of the model and its applicability to the cellular system.

For the 2-component model the probability for cell killing is

$$K = 1 - \exp(-D/D_1)\{1 - [1 - \exp(D/D_m)]^m\} \tag{6}$$

whose low dose limit is again linear with D, for

$$\lim_{D\to 0} K = D/D_1 \tag{7}$$

so that

$$S = K/(D/D_1) = \frac{1 - \exp(-D/D_1)\{1 - [(1 - \exp(-D/D_m)]^m\}}{D/D_1} \tag{8}$$

Here the supralinearity threshold varies with m. When D_1/D_m exceeds the value given below for a given m, the supralinearity index begins to exceed 1. Only for values in excess of those given for particular values of m is it possible for the RBE to exceed 1 for any high LET radiation, when the 2-component limit is used to represent the probability for cell killing by gamma rays. The thresholds are (m, D_1/D_m): (2, 1), (2.5, 1.5), (3, 1.8), (4, 2.3), (5, 2.5), (10, 3.8), and (20, 4.5).

These constraints cannot be violated by a theory of RBE, purporting to represent a homogeneous cell population, unless the difference in temporal distributions of ionizing events between low and high LET radiations is a central component of the theory.

4. Experimental findings

There are many sets of experimental results which violate this RBE constraint. Let us enumerate a few.

For murine leukemia cells *in vitro,* Caldwell, Lamerton, and Bewley (1965) found the RBE for neutrons to be 2.3, while the survival curve for X-rays was nearly exponential.

For *Shigella flexneri* grown in nutrient broth, Alper (1974) found an RBE of 1.8 for neutrons, on a system whose survival is nearly exponential after electron irradiation.

For chlorella cells irradiated with ^{60}Co gamma rays, at dose rates from 9 rad min^{-1} to 12,000 rad min^{-1}, deChoudens, Gilet, Roux, and Fabre (1975) report good fits to the $\alpha-\beta$ model, with α^2/β greater than 1.5 in all cases. All dose-response curves for gamma rays were sublinear. Yet the RBE reported for a variety of other radiations, from 650 keV electrons, 4–40 MeV protons, 1.38–4.5 MeV alpha particles, and 14 MeV neutrons all exceeded 1.

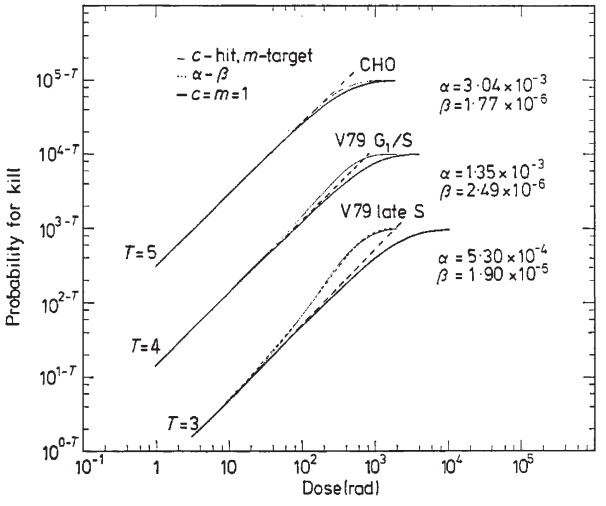
For CHO (G_1/S) cells, Hall (1975) found linear to sublinear response to gamma rays, with $\alpha^2/\beta = 5.2$, and slightly supralinear response, below saturation levels, for gamma rays for V79 (G_1/S) cells with $\alpha^2/\beta = 0.7$. Both cell lines displayed an

RBE greater than 1 for neutrons. Curiously, the RBE of the sublinear cell line was greater than the RBE of the slightly supralinear cell line.

Studies of the response of mouse intestine to fractionated doses of low LET radiations by Wambersie, Guelett, and Dutreix (1973) yielded the result that the "recovered dose" data are well fitted by the 2-component model, with parameters (D_1, D_m, m) lying in the neighborhood of (600, 300, 10) and (450, 225, 20), both sets of parameters being sublinear. Neutron irradiation of such cells customarily gives RBE > 1.

There are numerous illustrations of experimental violations of the supralinearity criterion detailed here. The question is not one of the experimental finding, but rather how it is to be interpreted.

Let us consider, as an illustration, the manner in which cell survival models can be fitted to the data of Hall (1975). These data can be fitted by an $\alpha-\beta$ model, preferred by Hall, by the 2-component model, illustrated by Hall, both on the assumption of a homogeneous cellular sample, and by both the multitarget and multihit models on the assumption of a heterogeneous sample. Some of these fits are illustrated in Figure 1. Note that the mixed systems include only a small fraction of 1-hit cells. Differences, between the curves calculated for the parameters given by Hall for the $\alpha-\beta$ model and those shown on the figure for either the mixtures of *c*-hit or *m*-target models, are so small that they cannot be shown in the drawing, for the topmost two curves. For the lower curve the *c*-hit or *m*-target



c-hit			*m*-target		
c	D_c	%	*m*	D_m	%
1	65	20	1	656	20
2	150	80	2	210	80

c-hit			*m*-target		
c	D_c	%	*m*	D_m	%
1	58	8	1	58	8
2	200	92	2	280	92

c-hit			*m*-target		
c	D_c	%	*m*	D_m	%
1	28	2	1	28	2
2	280	98	2	392	98

Figure 1. The kill probability plotted against the absorbed dose of ^{60}Co gamma rays, for CHO, V79 G_1/S, and V79 late S cells (Hall 1975). Plots from the $\alpha-\beta$ model fitted by Hall to his data are compared to a 45° dashed line matched at low dose to the data, and to a saturating exponential 1-hit curve (heavy line). Mixtures of *c*-hit (1 and 2 hit) and *m*-target (1 and 2 target) dose–response curves are also fitted to the data. Only for V79 late S cells are the fits of these curves distinguishable (dashed curve) from the $\alpha-\beta$ model (light curve). However, the 2-hit or the 2-target component of the mixtures are supralinear, and can plausibly be responsible for an RBE greater than 1 after neutron irradiation, while the sublinear or the barely supralinear fitted curves of the $\alpha-\beta$ model cannot.

curves are shown as a solid line just discernible, from the $\alpha-\beta$ model, shown as a dashed line. For all three cases a longer dashed 45° line is shown, as well as a saturating exponential curve, characteristic of the 1-hit detector. Unfortunately, Hall has given no results for the neutron irradiation of V79 late S cells, which show the most supralinear response to gamma rays. It is not a new result that cell survival data can be fitted by different models. It is a new result that a criterion exists such that a simpler model with 2 parameters (the $\alpha-\beta$ model) can be rejected in favor of a more complicated model (an *m*-target mix) on the basis of a comparison of low and high LET survival data.

But these results do not depend on mathematical models of cell survival data.

Once again, if one plots the probability for cell killing against the absorbed dose of gamma rays on a log-log plot, and compares the results to a 45° line drawn through the lowest dose point, an RBE greater than 1 is permitted for any high LET radiation field only if the plotted data lie above the 45° line. Contrary data require careful interpretation. It is not an unlikely inference that the cellular sample is heterogeneous. One must examine with some care the data marshaled in support of the view that the survival curves observed for mammalian cells after gamma irradiation have an initial negative slope rather than a shoulder, to examine whether these data are consistent with the present constraint.

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Résumé

Contraintes de transfert d'énergie linéaire élevé sur la survivance au faible transfert d'énérgie linéaire

Les courbes de survivance des cellules biologiques irradiées par des rayons gamma (et autres radiations à faible transfert d'énergie linéaire) ne peuvent pas être interprétées correctement sans examiner les implications de ces interprétations sur les radiations à transfert d'énergie linéaire élevé. La théorie d'efficacité biologique relative demande que cette efficacité pour tout champ de radiation à transfert d'énergie linéaire élevé est au

plus un lorsque la probabilité de destruction de la cellule en fonction de la dose de rayons gamma absorbée est linéaire ou sous-linéaire, pour des échantillons homogènes dont les paramètres de radiosensibilité ne sont pas modifiés pendant l'irradiation. Un résultat expérimental contraire suggère fortement que l'échantillon expérimental est hétérogène. La violation de la contrainte n'est pas exceptionnelle lorsque la courbe de survivance trouvée avec des radiations à faible transfert d'énergie linéaire possède une pente initiale négative bien définie.

Zusammenfassung

Zwangsbedingungen für das Überleben biologischer Zellen bei Bestrahlung mit Strahlen mit niedriger Linearenergie-Übertragung unter Berücksichtigung der Bestrahlung mit Strahlen mit hoher Linearenergie-Übertragung

Die Kurvenverläufe fur das Überleben biologischer Zellen, die mit Gammastrahlen (und anderen Strahlen mit niedriger Linearenergie-Übertragung) bestrahlt worden sind, lassen sich nicht richtig deuten, sofern man nicht die Bedeutung dieser Auslegungen für Strahlen mit hoher Linearenergie-Übertragung mitberücksichtigt. Die Theorie der relativen biologischen Wirksamkeit bedingt, dass die relative biologische Wirksamkeit einer Strahlung mit hoher Linearenergie-Übertragung im äussersten Fall den Wert eins annehmen kann, sofern die Wahrscheinlichkeit der Zellvernichtung als Funktion der aufgenommenen Gammstrahlendosis bei homogenen Mustern, deren Parameter für die Strahlenempfindlichkeit keiner Äderung während des Bestrahlungsvorgangs unterliegen, einen linearen bzw. sublinearen Verlauf annimmt. Ein experimenteller Befund mit gegenteiliger Aussagekraft gibt einen starken Anhaltspunkt dafür, dass das Versuchsmuster heterogen ist. Eine Durchbrechung der Zwangsbedingung tritt mit nicht ungewöhnlicher Häufigkeit dort auf, wo die bei Strahlen mit niedriger Linearenergie-Übertragung gefundene Kurve für das Überleben der Zelle eine klar ausgebildete negative Anfangsneigung besitzt.