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A CONVENIENT EXTENSION OF THE LINEAR-QUADRATIC MODEL TO INCLUDE REDISTRIBUTION AND REOXYGENATION

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Purpose: At present, the linear-quadratic model for cellular response to radiation can incorporate sublethal damage repair and repopulation. We suggest an extension, termed LQR, to include also the other two "Rs" of radiobiology, cell cycle redistribution, and reoxygenation.

Methods and Materials: In this approach, redistribution and reoxygenation are both regarded as aspects of a single phenomenon, which we term resensitization. After the first portion of a radiation exposure has decreased the average radiosensitivity of a diverse cell population by preferentially sparing less sensitive cells, resensitization gradually restores the average sensitivity of the population towards its previous value. The proposed LQR formula is of the same form as the original LQ formula, but with two extra parameters, an overall resensitization magnitude and a characteristic resensitization time. The LQR model assumes that resensitization is monotonic rather than oscillatory in time, i.e., always tends to increase average cellular sensitivity as overall time increases. We argue that this monotonicity assumption is likely to hold in clinical situations, though a possible extension is discussed to account for oscillatory decay of resensitization effects.

Results: The LQR model gives reasonable fits to relevant experimental data in the literature, reproducing an initial rise in cell survival, due to repair, as the treatment time is increased, followed by a resensitization-related decrease in survival due to redistribution and/or reoxygenation for treatment times of the order of the cell cycle time, and a final survival increase due to repopulation as the treatment time is increased still further.

Conclusion: The LQR model is a simple and potentially useful extension of the LQ model for computing more realistic isoeffect relations for early responding tissues, including tumors, when comparing different radiotherapeutic protocols.

Redistribution, Reoxygenation, Repair, Repopulation, Linear-quadratic, Isoeffect relations.

INTRODUCTION

There is increasing interest in the use of alternative fractionation schemes in radiotherapy, to increase the therapeutic advantage between tumor control and late sequellae (9). In parallel, there has been an increased focus on tools to predict isoeffect relations, when changes are made in dose, dose rate, fractionation, or overall time. Currently, the most commonly used tool is the linear-quadratic (LQ) approach (19, 42), which stems from a mechanistically based model for cell killing and sublethal damage repair (28). The LQ approach remains applicable in situation where repair between fractions is incomplete (42). An extension of the LQ model, the LQ + time model, which takes into account cellular repopulation, was first suggested by Travis and Tucker (45), and has subsequently been developed by several others (3, 10, 19).

Thus, among the "4 Rs" of radiobiology (Repair, Repopulation, Redistribution, and Reoxygenation) identified by Withers (51), the LQ + time model is currently capable of dealing with the first two. However, it is quite probable that cellular redistribution and, in some circumstances, tumor reoxygenation, affect the radiotherapeutic response to fractionated or continuous exposure (20).

Both redistribution and reoxygenation are complex phenomena and several multiparametric computer simulation calculations have been introduced in an attempt to

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understand their underlying mechanisms (14, 15, 25, 53, 55, 56). Although such approaches are important in elucidating and testing mechanisms, their complexity and multiparametric nature result in, as yet, limited application for the estimation of isoeffect relations in clinical practice.

We suggest here that both redistribution and reoxygenation potentially lead to the same type of outcome after the initial stage of a fractionated or prolonged exposure, namely a gradual increase in average cell population sensitivity towards its pretreatment level. Based on this observation, we discuss a convenient extension to the LQ formalism, which we term the LQR approach, because it now incorporates all four "Rs." The approach, although mechanistically driven, is sufficiently simple, and has sufficiently few extra parameters (two), to be potentially of practical use in radiotherapy. In a subsequent article, some proposed practical applications of the approach in radiotherapy will be discussed.

We first briefly review here the phenomena of redistribution and reoxygenation, further details being discussed, e.g., by Hall (20).

Redistribution

Radiation-induced redistribution (sometimes called reassortment) is the process whereby the proportion of cells in different phases of the cell cycle is altered by a radiation exposure and subsequent cell–cycle progression. Following the work of Elkind *et al.* (17) and many others, it is now clear that the first or early part of a radiation exposure of an asynchronous cycling cell population will preferentially spare cells that are in a resistant phase of the cell cycle. Subsequent progression of these resistant cells to a more radiosensitive part of the cell cycle will tend to decrease survival upon further irradiation at a later time.

This redistribution phenomenon is sharply demonstrated in "split-dose" experiments where the time between two exposures is progressively increased. As the time between fractions is increased up to a few hours, the surviving fraction increases due to sublethal damage repair. However, as the time between fractions is further increased beyond the few hours needed for full repair, up to times comparable to the cell cycle time, the surviving fraction decreases due to redistribution. In other words, resistant cells that preferentially survived the first fraction had sufficient time to move into a more sensitive part of the cycle, and were killed by the subsequent dose fraction. In addition to normal cell–cycle progression, radiation induced perturbations, such as G_2 delay in which cells may be temporarily arrested in a sensitive portion of their cycle, are often important components of redistribution.

The phenomenon of redistribution is probably ubiquitous in cycling cells, and has been observed both *in vivo* (1, 18, 23, 32, 36, 37, 44, 50, 52), and *in vitro* (16, 17, 29, 40), as well as in multicellular spheroids (41). It has also been demonstrated *in vitro* using continuous low dose rate irradiation (31, 33, 39).

Reoxygenation

Reoxygenation is the phenomenon by which hypoxic tumor cells surviving a first or partial radiation exposure can become increasingly oxygenated. Its potential clinical significance lies in the fact that tumors often contain a proportion of hypoxic cells that are less sensitive to radiation than corresponding well-oxygenated cells. Reoxygenation, first demonstrated by van Putten and Kallman (47), allows some of the surviving hypoxic cells to move into a more sensitive (oxic) state before a subsequent exposure. The time constant for this process varies from a few hours to a few days (20), with a variety of different metabolic processes being linked to the phenomenon (5).

Common features of redistribution and reoxygenation: Resensitization

Although *prima facie* quite different phenomena, redistribution, and reoxygenation do share a common outcome (43), a postirradiation increase in the sensitivity of cells that survive an initial or partial exposure. Following Hlatky *et al.* (22), we denote this common outcome *resensitization*. In general, resensitization occurs when (a) an early part of a radiation exposure leads to decreased average radiosensitivity just after the dose is administered, by preferentially killing the more radiosensitive cells of a diverse population; and (b) subsequent biologically driven changes gradually restore the original population average radiosensitivity. It is the general phenomenon of resensitization that we model here by an extension to the LQ model.

Rationale for the LQR model

As in the LQ model, the general rationale is to reproduce the main features of a complex situation using a minimum number of adjustable parameters. Thus, we do not attempt to analyze resensitization in detail by specific estimates of such complex effects as normal progression through the cell cycle, G_2 delay, or fluctuations in oxygen status; rather, we use a simpler model (22), involving one overall resensitization magnitude and one characteristic resensitization time, leading to a formalism virtually identical in mathematical structure to the existing LQ equations.

The essential assumption of the LQR model is that the effects of resensitization decrease monotonically with time, i.e., they tend to produce a continuous increase in average radiosensitivity back toward preirradiation values. This assumption must, in general, be an approximation that will not always be reasonable, particularly in highly homogeneous cell systems such as can be obtained *in vitro*. For example, cell–cycle synchrony due to preferential sparing of cells in resistant parts of the cycle followed by normal progression through the cycle, might

be expected to lead to oscillatory resensitization effects. Although we discuss (in Appendix A) a generalization of the LQR approach in which a monotonic change is replaced by damped oscillatory behavior, we argue that, in clinical practice, monotonicity is likely to be a reasonable assumption. Specifically, at any given time, cells in a tumor form a highly diverse population (21), differing from each other both genetically and epigenetically, as well as biochemically. The consequent distribution of properties such as cell–cycle time and oxygen status would be expected to average out oscillations in the process of resensitization (22).

Similar comments apply to radiation-induced delay. For example, Denekamp (11) quotes a typical G_2 delay time of ~1 h, or ~10% of the overall cycle time, per Gy. Recent results suggest that such values are generally reasonable (27, 34, 46, 49, 55). For therapeutically relevant fractions of ~2 Gy, such delays should be consistent with monotonic resensitization, because the number of extra cells in G_2 at any given subsequent time would not dominate the average response of a diverse cell population.

It is possible that more extreme fluctuations of population radiosensitivity could occur during fractionated radiotherapy. For example, G_2 delay is cell-line dependent (27, 34, 46, 49, 55), and a few cell lines show delays as large as one cell–cycle per Gy. Such long delays, if accompanied by extra G_2 radiosensitivity, could lead to situations where the average radiosensitivity temporarily rises to levels greater than preirradiation values, after an acute dose of several Gy. If such drastic fluctuations in radiosensitivity occur, they would be beyond the scope of the LQR model and the generalization given in Appendix A. However, we know of no data that demonstrate such major increases in sensitivity in a heterogeneous cell population.

Although not a necessary aspect of the LQR model, we shall assume here that resensitization can be described by one average characteristic resensitization time, and a corresponding single resensitization amplitude. This assumption is reasonable if any one of the biological processes underlying resensitization is dominant, or if the various processes that drive resensitization have comparable time scales. Again, this assumption should be considered as an approximation, analogous for example to the practical use of a single characteristic time for sublethal damage repair, even though this single repair time is derived from a variety of underlying repair processes. Although biexponential kinetics could be used, the extra parameters that would be introduced make this an undesirable option.

METHODS AND MATERIALS

The essential assumption of the model is that resensitization can be described by a single amplitude and a single characteristic time. An earlier presentation (22) of the proposed LQR (i.e., extended LQ) formalism was structured in terms of a partial differential equation containing the resensitization amplitude and time as parameters. We give here a simpler description that is equivalent and easier to use.

Before discussing the LQR model for general dose-delivery protocols, we will discuss two special cases that illustrate the principles underlying this repair + resensitization approach. The first special case concerns the response of a diverse cell population to a single acute dose, and the results here coincide with those for a Gaussian model suggested by Schultheiss *et al.* (38). The second special case, of two acute doses separated by an adjustable time interval, illustrates the time dependence in the LQR model. After describing these two special cases, we describe the LQR model for the general case, where any temporal pattern of dose delivery is allowed.

Special case I: Response of a heterogeneous cell population to acute irradiation

Schultheiss *et al.* (38) suggested a Gaussian probability model for analyzing the response of diverse cell populations to an acute radiation exposure. Of interest here is a special case that can be described as follows: let us assume that a cell population is composed of various subpopulations, i, with different sensitivities, and that each subpopulation responds to a single acute dose, D, according to the LQ dose–response relation:

$$S_i = \exp[-\alpha_i D - \beta D^2]. \qquad \text{(Eq. 1)}$$

For example, the different subpopulations might correspond to cells in different stages of the cell cycle, or with different levels of oxygenation, or both. We assume that the value of β is effectively the same for all subpopulations (as discussed in Appendix C, the effects of fluctuations in the parameter β are likely to be small). Finally, we shall assume that, prior to an acute exposure to a dose D, the probability distribution of the random variable, $\boldsymbol{\alpha}$, corresponding to the linear parameter in Eq. 1, is Gaussian, characterized by an average value α and variance σ^2. Then, averaging Eq. 1 over the diverse cell population gives the following two main results (22, 38): first, the surviving fraction for the overall population is

$$S = \exp[-\alpha D - (\beta - \tfrac{1}{2}\sigma^2)D^2]. \qquad \text{(Eq. 2)}$$

Comparing Eq. 2 with the standard LQ Eq. 1, there is an extra term $\frac{1}{2}\sigma^2 D^2$. This term corresponds to an increase in the surviving fraction due to cell-to-cell diversity. Its interpretation is that the extra resistance of particularly resistant cells "outweighs" the extra sensitivity of particularly sensitive cells. Equation 2 implies that if the quadratic (dose squared) term is measured by generating a

dose–response curve for acute exposures, the coefficient obtained will be $\beta - \frac{1}{2}\sigma^2$ (38). Some experimental evidence for the situation implied by Equation 2 has been described elsewhere (13, 22, 30, 38).

In addition to Eq. 2, the second main prediction of the Gaussian model is that, immediately after an acute irradiation, the distribution of the random variable, $\boldsymbol{\alpha}$, remains Gaussian and has the original variance σ^2, but its average value is decreased. Specifically, the new value, averaged over the surviving cells, is $\alpha - \sigma^2 D$. The interpretation of this decrease in the average value of $\boldsymbol{\alpha}$ corresponds to that originally suggested by Elkind *et al.* (17): resistant cells are preferentially spared, so that just after irradiation they comprise a larger fraction of the population than before irradiation. This larger fraction of resistant cells corresponds to a decrease in radiosensitivity.

The arguments that we have made here, following those of Schultheiss *et al.* (38), are based on the assumption that $\boldsymbol{\alpha}$ is normally distributed. In fact we show, in Appendix B, that Eq. 2 remains valid at doses of relevance to radiotherapy, even in the non-Gaussian case.

Special case II: The LQR repair/resensitization model for two acute fractions

The standard LQ model deals with time dependence by using a repair time τ_R, which is of the order of minutes or hours. The LQR model additionally uses an average resensitization time τ_S, which would be expected to have an order of magnitude comparable to cell cycle times or reoxygenation characteristic times, i.e., hours to days. Roughly speaking, the extra population resistance that occurs just after an acute irradiation, due to preferential sparing of resistant subpopulations, is postulated to die away monotonically on the time scale τ_S, as biological processes such as cell–cycle redistribution or reoxygenation alter the resistance of individual cells.

We next give results of the LQR repair/resensitization model in a special case illustrating the main results of the general case. Suppose two acute exposures, D_1 and D_2, are separated by a time T. Then the predicted surviving fraction is (22)

$$S = \exp[-\alpha(D_1 + D_2) - \beta(D_1^2 + D_2^2 + 2D_1D_2\, e^{-T/\tau_R}) + \tfrac{1}{2}\sigma^2 (D_1^2 + D_2^2 + 2D_1D_2\, e^{-T/\tau_S})], \quad \text{(Eq.3)}$$

where α is again a population average prior to irradiation. The terms involving α and β are the standard ones of the LQ model including incomplete repair (4, 19, 42). The time-independent terms involving σ^2 have been discussed above, and refer to an increase in survival due to cell-to-cell heterogeneity. Thus, the only novelty in Eq. 3 is the last term, $+\sigma^2 D_1 D_2 \exp(-T/\tau_S)$, which is a time-dependent resensitization term: as the interfraction interval T increases, this term tends to zero, and its disappearance makes the survival decrease, i.e., resensitization effects tend to decrease survival at larger times T. Of course, there is another time-dependent term in Eq. 3, $-2\beta D_1 D_2 \exp(-T/\tau_R)$, corresponding to sublethal damage repair, and this term, having the opposite sign, has the opposite influence. It is the balance between these two effects (as well as repopulation, discussed below) that results in the characteristic shape of the "split-dose" survival curve pointed out by Elkind *et al.* (17) and others.

The LQR repair/resensitization model for arbitrary fractionation schemes

Suppose a radiation protocol, either fractionated or continuous, delivers a total dose D during a total time T. If the assumptions of the LQR repair/resensitization model hold then, in general,

$$S = \exp[-\alpha D - \beta G(\tau_R)\, D^2 + (\tfrac{1}{2}\sigma^2)\, G(\tau_S)\, D^2]. \quad \text{(Eq. 4)}$$

Here, the first term describes cell killing by one-track action, the second term describes killing by two-track action (and possible repair), while the third term refers to intercellular diversity of radiosensitivity and resensitization. Here $G(\tau)$ is the generalized Lea-Catcheside function defined (4) by

$$G(\tau) = \left(\frac{2}{D^2}\right) \int_0^T du\, R(u) \int_0^u dw\, R(w)\Phi(u, w);$$

$$\Phi(u, w) = \exp[-(u - w)/\tau], \quad \text{(Eq. 5)}$$

where the function $R(t)$ describes the variation in dose rate as a function of time over the entire course of the treatment. By choosing $R(t)$ appropriately, any radiotherapeutic protocol can be considered. For example in a continuous low dose rate protocol, the function $R(t)$ is a constant (D/T), and substituting this into Eq. 5 gives the Lea-Catcheside function (28):

$$G(\tau_R) = 2(\tau_R/T)^2 (e^{-T/\tau_R} - 1) + 2\tau_R/T,$$
$$G(\tau_S) = 2(\tau_S/T)^2 (e^{-T/\tau_S} - 1) + 2\tau_S/T. \quad \text{(Eq. 6)}$$

Note that the resensitization terms involving $\frac{1}{2}\sigma^2$ in Eqs. 3 and 4 are formally identical to the damage/repair terms involving β, apart from different values of the characteristic time and amplitude, and the (crucial) change in sign. The mathematical proof of Eqs. 3 and 4 is based on showing that the distribution function for the random variable $\boldsymbol{\alpha}$ is Gaussian, with a fixed variance at all times; it follows that the time development of the distribution function is determined by specifications of how the average value of $\boldsymbol{\alpha}$ changes with time. The details are an extension of calculations given earlier (4, 22).

Repopulation

In most situations where resensitization is potentially of importance, the total exposure time is comparable to, or greater than, the cell cycle time. Thus, it is important to take into account cellular repopulation. In the following, this is done using the simple exponential growth model proposed by Travis and Tucker (45), in which a time-dependent exponential term is factored into the predicted survival:

$$S(D) \Rightarrow S(D)\, e^{T/\tau_P} \qquad \text{(Eq. 7)}$$

where T is the overall exposure time, and τ_P is a characteristic time for cellular or tumor repopulation. (More complex approaches in place of this simple exponential model have been considered (3, 19), and could be used if desired, but will not be considered further here.) Here, for example, the LQR formula for the special case (Eq. 3) of a split-dose experiment becomes

$$S = \exp[-\alpha(D_1 + D_2) - \beta(D_1^2 + D_2^2 + 2D_1D_2\, e^{-T/\tau_R}) + \tfrac{1}{2}\sigma^2 (D_1^2 + D_2^2 + 2D_1D_2\, e^{-T/\tau_S}) + T/\tau_P] \qquad \text{(Eq. 8)}$$

and the general LQR formula, Eq. 4, becomes

$$S = \exp[-\alpha D - \beta G(\tau_R)\, D^2 + (\tfrac{1}{2}\sigma^2)\, G(\tau_S)\, D^2 + T/\tau_P], \qquad \text{(Eq. 9)}$$

As before, in both these equations the first term describes cell killing by one-track action, the second term describes killing by two-track action (and possible repair), and the third term refers to resensitization; the final term now describes repopulation.

RESULTS

Here, we apply the LQR formalism to the analysis of laboratory-based experiments. (Clinical applications will be discussed in a subsequent article.) There are a variety of experiments that would be appropriate for analysis with the formalism described here. In particular, "split-dose" experiments, in which two fractions are separated by increasing times, are capable of exhibiting the effects of redistribution and, in appropriate situations, reoxygenation. We make no attempt to analyze all appropriate data, but consider a few representative experiments, on the basis of which the advantages and problems of the LQR model will be discussed.

Data analysis was performed by fitting experimental results to Eq. 8, using the technique of simulated annealing (26). Simulating annealing is a useful curve-fitting technique when the fitted function is sufficiently complex that the minimization search procedure can otherwise easily fall into "false minima"; by sometimes taking "uphill" steps in the search, simulated annealing potentially avoids such problems.

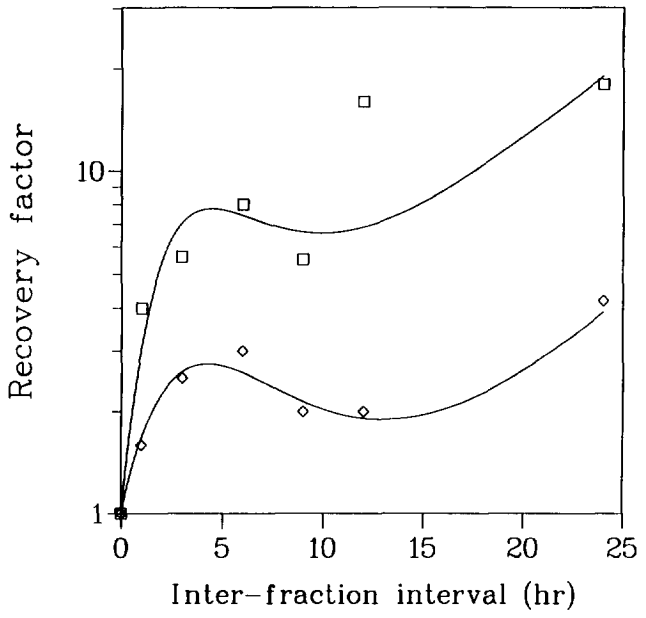

Fig. 1. Modeling combined effects of repair, resensitization, and repopulation. Data points are from Belli *et al.* (1) for P-388 tumors exposed *in vivo* to two acute doses separated by the time shown on the horizontal axis. Recovery factor is the ratio of clonogenic survival to that for zero time separation. ◇ and lower curve: hypoxic tumors exposed to 15 Gy + 15 Gy. □ and upper curve: aerobic tumors exposed to 5 Gy + 5 Gy. The curves are from the LQR model, Eq. 8, with the parameter choices shown in Table 1. For small interfraction times there is a characteristic rise of recovery factor, attributed to repair of sublethal lesions; at larger times there is a decrease, attributed in the model to resensitization, the final rise then being attributed to repopulation.

Figure 1 shows fits of Eq. 8 to the data reported by Belli *et al.* (1), which are the results of split-dose experiments with either oxic or hypoxic P388 lymphocytic tumors in the mouse. The features discussed above are clearly present here, with an initial rise in cellular survival due to sublethal damage repair, a subsequent decrease due to resensitization (redistribution/reoxygenation), followed by a final rise, assumed (see below) to be primarily due to repopulation. Figure 2 (solid curve) shows corresponding fits of Eq. 8 for an *in vivo* split-dose experiment reported by Till and McCulloch (44) on marrow cells proliferating in mice. (The dashed curve in this and subsequent figures, referring to a generalized model, are discussed below and in Appendix A.) Figure 3 shows fits to survival assayed *in vitro* for human HeLa cells exposed to a split-dose regime (29).

It is apparent from Figs. 1–3 that the formalism described here does reproduce the main trends in cellular survival or isoeffect relations as a function of overall time. However, Fig. 4 shows some further split-dose data, for V-79 cells (17), where the measured survival increases by more than a factor of 6 when the overall time is in-

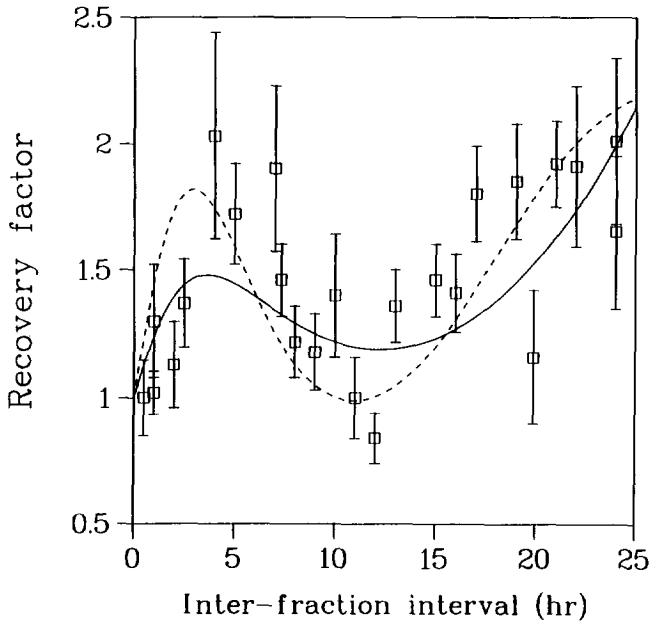

Fig. 2. Data points are from Till and McCulloch (41) for mouse marrow cells exposed *in vivo* to two acute doses (2 Gy + 2 Gy) separated by the time shown on the horizontal axis. Recovery factor is the ratio of clonogenic survival to that for zero time separation. The solid curve is from the LQR model, as in Fig. 1. The dotted curve is for a generalization of the LQR model, described in the Appendix A, which has one extra adjustable parameter. The parameter choices are given in Table 1.

creased from 5.6 to 9.5 h. In the LQR formalism this final increase in survival, occurring after the minimum produced by resensitization, is taken to be entirely due to repopulation; this follows from the exponential term, $\exp(-T/\tau_S)$, in Eqs. 8 and 9, which implies that resensitization causes strictly a monotonic decrease in survival with increasing time. An increase in survival of a factor of 6 in 4 h is too fast to be attributable solely to repopulation. Reference to Fig. 2 might also suggest a faster increase immediately after the survival minimum than could be accounted for solely on the basis of repopulation.

The inability of the LQR model to reproduce a very rapid rise in survival sometimes seen (Figs. 2 and 4, but not Figs. 1 and 3) immediately after the resensitization minimum, implies that resensitization effects do not always change in a strictly monotonic way with increasing time. Indeed, one might expect (17) that if an initial dose causes some synchronization in a resistant part of the cell cycle, increasing time will cause those cells to move into a more sensitive part of the cell cycle (resensitization), but still more time would allow them to revert to a resistant phase (desensitization).

In a heterogeneous situation, such as a human tumor, the effects of the initial partial synchrony will be lost quite rapidly. Then the effects of a post-resensitization *desensitization* might be expected to be small, and the assumption in the LQR model that resensitization causes a monotonic decrease in survival with time is likely to be adequate. However, in Appendix A, we discuss a further modification of the LQR model, essentially a generalization of Eqs. 8 or 9, in which the assumption that the effects of resensitization are strictly monotonic with time is relaxed. Specifically, cell-cycle related arguments imply that the time dependence of the resensitization term may be generalized from $\exp(-T/\tau_S)$ to $\exp(-T/\tau_S)\cos(\omega T+\phi)$, where the phase constant, ϕ, is fixed by external mechanistic arguments. This latter function, which contains one further parameter (ω), describes damped oscillations, rather than a continuous decrease with increasing overall time. This generalized formalism can model a rapid rise in cellular survival after the resensitization minimum, now assumed to be related both to repopulation *and* desensitization due to redistribution. Examples are shown by the dashed-curve fits in Figs. 2 and 4.

DISCUSSION

It has long been a valid criticism of the application of the LQ model to radiotherapy that the model takes into account only some of the factors affecting radiotherapeutic response to fractionated exposure; specifically, it takes into account sublethal damage repair and, in a later modification, repopulation, but not redistribution or reoxygenation. We have suggested that both redistribution and reoxygenation exhibit a similar common outcome, namely resensitization. Resensitization (22) occurs when a radiation exposure preferentially kills radiosensitive cells in a

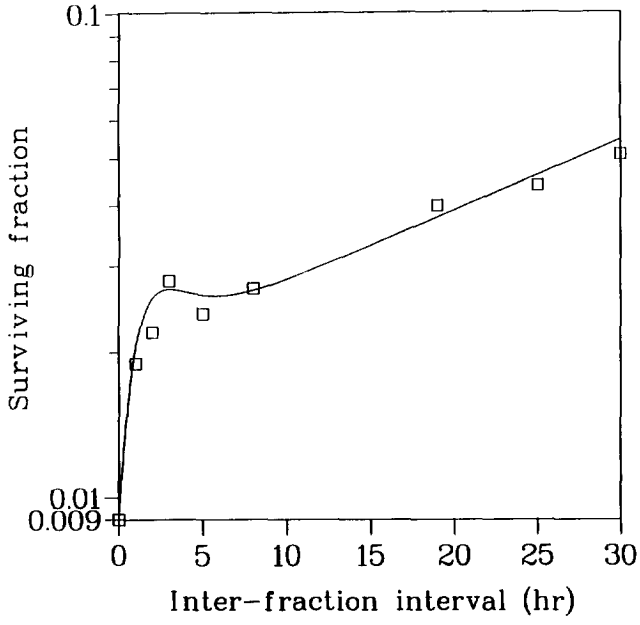

Fig. 3. Data points are from Lockart *et al.* (26) for clonogenic survival of HeLa cells exposed *in vitro* to two acute doses (2.71 Gy + 3.61 Gy) separated by the time shown on the horizontal axis. The curve is from the LQR model, as in Fig. 1. The parameter choices are given in Table 1.

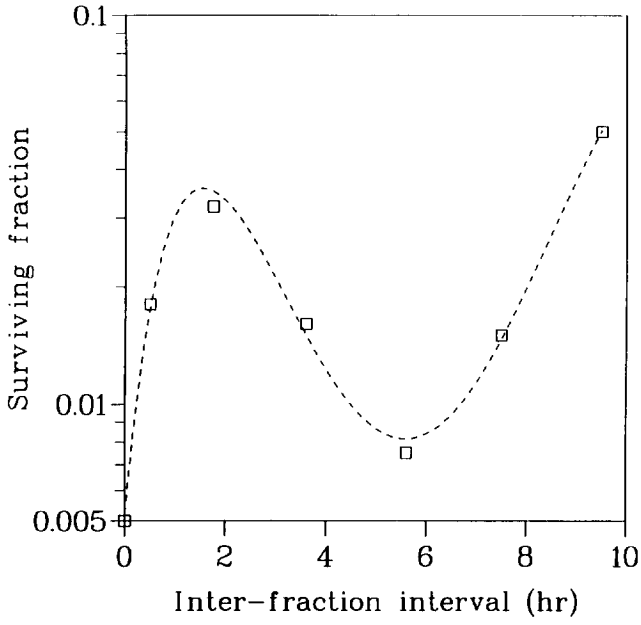

Fig. 4. Data points are from Elkind *et al.* (16) for clonogenic survival of V-79 cells exposed *in vitro* to two acute doses (7.47 Gy + 8.04 Gy) separated by the time shown on the horizontal axis. The dotted curve is, as in Fig. 2, for the generalized LQR model (Appendix A), with parameters shown in Table 1. In this case, a fit using the LQR model itself gives unrealistically small values for the characteristic repopulation time.

diverse population, producing a decreased average radiosensitivity just after the dose is administered; after this radiation exposure, biologically driven changes then tend to restore the original population average radiosensitivity. We have proposed a simplified repair/resensitization model, termed LQR, which extends the LQ model to take into account the combined effects of redistribution and reoxygenation.

The LQR model represents an extension to the LQ + time model. In terms of parameter numbers, the standard LQ model has a total of three parameters (α, β, τ_R), the simplest extension to include repopulation has one more (τ_P), and the LQR extension proposed here has two further parameters (resensitization characteristic time and resensitization amplitude). In terms of mathematical complexity, the LQR model is essentially the same as the LQ model—a second "dose-squared" term being added, which is of exactly the same structure (though with a different sign) as the dose-squared term in the standard LQ equation.

Parameters of the model were evaluated by comparison with some *in vitro* and *in vivo* experiments. The basic features of the experimental results, namely an initial increase in survival due to sublethal damage repair, followed by a decrease due to resensitization, followed by an increase due to repopulation, could generally be well reproduced using the LQR formalism. The LQR model predicts a quadratic increase in the effects of resensitization, as well as sublethal damage repair, with increasing dose. We do not know of any data that can be used to confirm unequivocally the predicted dose dependence of resensitization; for example, the results reported by Ruifrok *et al.* (37) using a mouse-skin model, while consistent with a quadratic dependence for resensitization, are also consistent with a linear dose dependency.

Although the original derivation of the LQR repair/resensitization model is quite complex, the key assumption involved is relatively simple: beyond the assumptions of the normal LQ + time model for repair and repopulation, the LQR model is based on the assumption that resensitization occurs monotonically, so that once sublethal damage repair is essentially complete, the average radiosensitivity of the cell population gradually increases towards its preirradiation value. For situations where such monotonic resensitization is present, the LQR model should prove useful, despite its inherent idealizations.

In some of the laboratory-based experiments analyzed here, it is clear that the assumption of monotonicity does not completely hold. We have discussed (Appendix A) a possible generalization of the LQR model that allows the effects of resensitization to undergo damped oscillations in time. The price of this generalization is a further free parameter. We have suggested that in a highly heterogeneous situation such as a human tumor, treating the effects of redistribution and reoxygenation as varying monotonically with time will be an adequate approximation. Our reasoning is that oscillations corresponding, for example, to synchronous progression through the cell cycle or to G_2 delay, are likely to be of small amplitude due to cellular diversity, differences in cycling status, differences in oxygenation, and other microenvironmental factors. Further analysis of this point is clearly needed.

Resensitization effects would be expected to apply most directly to tumors or early responding normal tissues, containing many rapidly cycling cells. Because typical time constants for resensitization (redistribution and/or reoxygenation) are quite long (hours to days), the LQR model may apply to most external beam radiotherapy protocols. Thus, with the caveats discussed above about resensitization monotonicity, the LQR model would be applicable not only to hyperfractionation, but also to conventional external beam radiotherapy delivering ~2 Gy/day, where the significance of redistribution and reoxygenation has long been debated (20, 51).

The applicability of the model to continuous low dose rate brachytherapy is less clear. Specifically, it may be that for therapeutically relevant dose rates, there is virtually complete cessation of cycling, due to delays at cell cycle checkpoints (33, 55). A situation where cycling essentially stops, and overall radiosensitivity is dominated by sensitivity in the particular states in which cells are arrested, would appear to be beyond the scope of the current approach.

Finally it is emphasized that the LQR model, while mechanistically driven, is designed to be sufficiently sim-

ple that it can be practically applied to isoeffect calculations in radiotherapy. The many idealizations in the consideration of resensitization parallel those inherent in the standard LQ model relating to repair. For example, while more complex multiparametric or simulation models have been developed to understand the detailed mechanisms of sublethal damage repair (2), these are not of direct practical use in radiotherapy. Similarly, complex models have been developed to understand the details of redistribution (14, 15, 25, 53, 54, 56) and reoxygenation (12). Ultimately, it might be anticipated that such approaches, particularly Monte-Carlo based simulation models, will be incorporated into biologically based treatment planning. However, we suggest that in the near future, comparatively simple analytic models of the type described here may be of more practical utility in the design of improved fractionation schemes.

APPENDIX A

Decay of resensitization through damped oscillations

We discuss a possible generalization of the LQR model discussed in the main text. Specifically, we wish to relax the assumption, inherent in the LQR model, that resensitization is monotonic with increasing time, leading to a steady increase in radiosensitivity. In this Appendix, the general picture is that the main resensitization effects are due to cell–cycle progression, and we, therefore, take advantage of standard detailed mathematical models of cell–cycle progression during the periods when no irradiation occurs.

Standard cell–cycle models typically address the cell number density function $n(a,t)$, where a is age, i.e., the time since mitosis of the parental cell. By definition, $n(a,t)da$ is the number of cells of age a in the range da at time t. These models are based on the von-Foerster partial differential equation, its discreet-time analogs, and its generalizations (35, 48). They predict that, in the absence of radiation, the cell number density function has the form (7)

$$n(a, t) = e^{\lambda t}[Cs(a) + s_1(a)e^{-\nu_1 t}\cos(\omega_1 t + \phi_1) + s_2(a)e^{-\nu_2 t}\cos(\omega_2 t + \phi_2) + \ldots]. \quad \text{(Eq. A.1)}$$

Where growth inhibition is not important, λ is the growth rate for exponentially growing cells, sometimes referred to as the "Malthusian" parameter (35); in simple situations, in the notation of the Methods and Materials section, $\lambda = 1/\tau_P$. In Eq. A.1, C is a constant and $s(a)$ is the "stable" cell population probability distribution, with $\int s(a)da = 1$. In the generic case, $0 < \nu_1 < \nu_2 < \ldots$, so that, for large times, the cell population pattern approaches the log-phase probability distribution $s(a)$, regardless of the cell population pattern at time $t = 0$. The rate of approach to the log-phase probability distribution is governed by ν_1, large values of ν_1 corresponding to rapid loss of cell–cycle synchronization. Detailed expressions for λ, A, $s(a)$, $s_i(a)$ ($i = 1, 2, 3, \ldots, \infty$), ν_i, ω_i, and ϕ_i depend on the detailed cell–cycle model and on initial conditions, with ω_1 having order of magnitude $2\pi/\tau_P$ (7). We will not need these detailed expressions in the arguments that follow. It is of interest to note that Eq. A.1 is also valid for density-inhibited populations, with $\lambda = 0$, for small deviations from equilibrium (35).

We consider a population of cells with age-dependent radiation sensitivity $\alpha(a)$, where α is the linear coefficient of the LQ model (see Eq. 1). Then the population average value is

$$\bar{\alpha}(t) = \frac{\int_0^\infty \alpha(a)n(a, t)da}{\int_0^\infty n(a, t)da} \quad \text{(Eq. A.2)}$$

Using Eqs. A.1 and A.2, and neglecting terms that decrease more rapidly than $\exp(-\nu_1 t)$, gives

$$\bar{\alpha}(t) = \alpha - \beta \exp(-t/\tau_S)\cos(\omega t + \phi) \quad \text{(Eq. A.3)}$$

where, again, α is the average over the unirradiated population, i.e.,

$$\alpha = \int_0^\infty \alpha(a)s(a)da. \quad \text{(Eq. A.4)}$$

In Eq. A.3, $\omega = \omega_1$, B is a constant, and, using the notation of the main text, $\nu_1 = 1/\tau_S$. Thus, during periods where no irradiation occurs, the average value of the random variable, $\boldsymbol{\alpha}$, gradually approaches α, the dominant correction term at large times being oscillatory and exponentially decaying.

The basic mathematical approximations of the generalized model are to approximate the probability distribution of the random variable, $\boldsymbol{\alpha}$, as a Gaussian with fixed variance σ^2, and to extrapolate its average value (Eq. A.3) to small times. For a single acute dose D delivered at time $t = 0$, the Gaussian approximation again implies that, just after $t = 0$, the average value of $\boldsymbol{\alpha}$ is decreased to $\alpha - \sigma^2 D$; the second approximation implies that for $t \geq 0$,

$$\bar{\alpha}(t) = \alpha - f(t)\sigma^2, \quad \text{(Eq. A.5)}$$

where

$$f(t) = A \exp(-t/\tau_S)\cos(\omega t + \phi), \quad \text{with} \quad A \cos \phi = 1. \quad \text{(Eq. A.6)}$$

A resensitization expression that incorporates the single acute-dose behavior shown in Eqs. A.5 and A.6, but is applicable to an arbitrary dose pattern, can be obtained by replacing $G(\tau_S)$ in Eq. 9 by the double integral in Eq. 5 with

Table 1. Parameter values obtained from fits of LQR model (Eq. 8) or generalized LQR model (Appendix A) to data in Figs 1–4

		α (Gy^{-1})	β (Gy^{-2})	$\frac{1}{2}\sigma^2$ (Gy^{-2})	τ_R (h)	τ_S (h)	$2\pi/\omega$ (h)	τ_P (h)
Fig. 1	Aerobic tumor cells *in vivo*, Belli *et al.* (1)		0.553	0.547	2.88	3.32		9.11
Fig. 1	Hypoxic tumor cells *in vivo*, Belli *et al.* (1)		0.1713	0.176	4.65	4.93		7.25
Fig. 2	Marrow cells *in vivo*, Till & McCulloch (41)		3.63	3.83	4.74	5.16		11.0
Fig. 2	Marrow cells *in vivo*, Till & McCulloch (41) (generalized LQR)		0.172	0.158	2.96	9.53	24.1	43.8
Fig. 3	HeLa cells *in vitro*, Lockart *et al.* (26)	0.490	2.14	2.10	1.46	1.50		29.8
Fig. 4	V-79 cells *in vitro*, Elkind *et al.* (16). (generalized LQR)	0.159	0.0223	0.0107	0.794	56.2	11.4	26.0

$\frac{1}{2}\sigma^2$ is the amplitude of resensitization effects, τ_S is the characteristic resensitization time, ω is the oscillation frequency for the generalized LQR model (Appendix A), and τ_P is the characteristic repopulation time. In some cases, when the data comprise only a ratio of effects, the α coefficient is not needed and cannot be determined from the published data.

$$\Phi(u, w) = f(u - w), \qquad \text{(Eq. A.7)}$$

where the function f is given in Eq. A.6. The monotonic LQR resensitization model used in the main body of the article can be obtained by taking the limit of $\omega \ll \nu$ in Eq. A.6.

For example, in the case of two doses, D_1 and D_2, separated by a time T, using Eq. A.7, the survival can be written

$$S = \exp[-\alpha(D_1 + D_2) - \beta\,(D_1^2 + D_2^2 + 2D_1D_2\,e^{-T/\tau_R}) + \tfrac{1}{2}\sigma^2\,(D_1^2 + D_2^2 + 2D_1D_2\,A\,e^{-T/\tau_S}\cos(\omega T + \phi))], \qquad \text{(Eq. A.8)}$$

where $A\cos\phi = 1$. Setting $\omega = 0$ in Eq. A.8 yields the LQR model of Eq. 3.

Using Eq. A.8, gives excellent fits to most of the data sets, though this is hardly surprising because there are now two extra adjustable parameters (ω and ϕ) beyond the already significant number used in the LQR model described in the main text. The number of additional parameters can be reduced by using the following argument for determining the phase, ϕ:

In a detailed model of resensitization caused by redistribution, Chen *et al.* (8) found that after an acute dose administered at $t = 0$, $(d\bar{\alpha}/dt)(0) = 0$. This condition is related to the fact that during cell cycle progression some cells will go from more sensitive to less sensitive states, even when the dominant trend is in the other direction. Thus, a plausible restriction on $f(t)$ in Eq. A.6 is $(df/dt)(0) = 0$, i.e., $\tan\phi = -1/(\omega\tau_S)$.

With this additional restriction, resulting in a model that has only one more parameter than the LQR model used in the main body of the article, good fits to the data can be obtained. It is these fits that are shown by the dashed curves in Figs. 2 and 4. The corresponding values of the various parameters (Table 1) are plausible, considering the mechanistic interpretations that the model assigns to the parameters.

APPENDIX B

Applicability of LQR formalism at low doses for non-Gaussian distributions

Like the model of Schultheiss *et al.* (38), the LQR model assumes a Gaussian distribution for $\boldsymbol{\alpha}$. We show here that, for sufficiently low doses, many of our considerations apply also to the non-Gaussian case. This generalization is relevant because Gaussian models of population response have well-known limitations (6, 24). For example, the occurrence of formally negative values of a must be taken into account (22).

For small doses, the relation given in Eq. 2 between the variance of the random variable $\boldsymbol{\alpha}$ and surviving fraction remains valid in the non-Gaussian case. In fact, using a Taylor series in dose D gives the following mathematical identity:

$$\ln\langle\exp(-\boldsymbol{\alpha}D)\rangle = -\alpha D + \tfrac{1}{2}\sigma^2D^2 - \tfrac{1}{6}\langle(\boldsymbol{\alpha} - \alpha)^3\rangle D^3 + O(D^4) \qquad \text{(Eq. B.1)}$$

Here $\langle\ \rangle$ denotes population averaging prior to irradiation; thus, $\alpha = \langle\boldsymbol{\alpha}\rangle$ is the average preirradiation value of $\boldsymbol{\alpha}$, $\sigma^2 = \langle(\boldsymbol{\alpha} - \alpha)^2\rangle$ is the variance, and $O(D^4)$ refers to higher-order terms. For the Gaussian case, Eqs. 1 and 2 of the text show that the same result, Eq. B.1, holds, with all the terms cubic or higher in D vanishing. Thus, for doses sufficiently small that terms cubic or higher in dose can be neglected, Eq. 2 would hold even if the distribution of $\boldsymbol{\alpha}$ is not Gaussian.

To gauge the significance of the higher order, non-Gaussian terms in Eq. B.1, we estimate the cubic term for a specific example. The value of $\langle(\boldsymbol{\alpha}-\alpha)^3\rangle$ depends on the details of the population diversity, but some insight is obtained by assuming a population with two-thirds of

its cells having a sensitivity half as large as average, and the remaining cells twice as sensitive as the average. In other words, for illustrative purposes, we consider a strongly bimodal population whose sensitivity varies by a factor of 4. In this case, an explicit calculation gives:

$$\tfrac{1}{2}\sigma^2 = \tfrac{1}{4}\alpha^2, \quad \tfrac{1}{6}\langle(\boldsymbol{\alpha} - \alpha)^3\rangle = \tfrac{1}{24}\alpha^3 \qquad \text{(Eq. B.2)}$$

Equation B.2 implies that, in this illustrative case, the D^3 term in Eq. B.1 is negative, corresponding to more killing than in a Gaussian model with the same mean and variance. The ratio of this D^3 term to the term αD is $(\alpha D)^2/24$. Using typical values (D = 2 Gy, α= 0.3 Gy^{-1}), the cubic term in Eq. B.1 represents a correction of only 1.5% to the dominant linear term. We conclude that for a typical fractionation protocol and reasonable $\boldsymbol{\alpha}$ values, Eq. 2 of the Methods and Materials section remains a good approximation, even if the distribution of $\boldsymbol{\alpha}$ is not Gaussian.

With regard to Eq. 3, describing survival after a split dose with time interval T, the equation remains valid in the limit as $T \Rightarrow 0$ in the non-Gaussian case, provided the dose is sufficiently low that cubic terms of Eq. B.1 can be neglected. This result can be seen by noting that in the limit as $T \Rightarrow 0$, two acute doses D_1 and D_2, which are administered in rapid succession, are equivalent to a single acute dose $D_1 + D_2$, whence Eq. B.1 applies. The validity of Eq. 3 as $T \Rightarrow 0$ implies that it is consistent to postulate (ad hoc) the remaining equations of the LQR model, as given in the Methods and Materials section of the article.

Finally, we note that the advantages and drawbacks of Gaussian models have been discussed elsewhere in the context of quantitative genetics (6, 24). Like cell survival models for protracted radiation protocols, quantitative genetics is concerned with the repeated selective elimination of subpopulations within a diverse population. The main considerations of quantitative genetics, regarding the average response and remaining variance in a surviving population, are surprisingly similar to the considerations of the LQR model. Generally speaking, quantitative genetics suggests that Gaussian models can handle the main effects (6) but need modification in special situations (24), and we suggest the same holds in the context of radiotherapy.

APPENDIX C

Effects of variations in $\boldsymbol{\beta}$ amongst subpopulations

In this Appendix, we show that, for fraction sizes comparable to 2 Gy, the effects of variations in $\boldsymbol{\beta}$ amongst subpopulations, neglected in our treatment, but considered by Schultheiss *et al.* (38), should be comparatively small for early responding tissues. The argument holds, irrespective of whether the joint distribution of $\boldsymbol{\alpha}$ and the random variable $\boldsymbol{\beta}$ is bivariate normal, as assumed by Schultheiss *et al.* (38).

Specifically, in analogy to the Taylor expansion of $\ln\langle\exp(-\boldsymbol{\alpha}D)\rangle$, given in Eq. B.1, the Taylor expansion of $\ln\langle\exp(-\boldsymbol{\alpha}D - \boldsymbol{\beta}D^2)\rangle$ gives all the terms in Eq. B.1 and the following extra terms:

$$-\langle\boldsymbol{\beta}\rangle D^2 + \rho\sigma\sigma_\beta D^3 + O(D^4), \qquad \text{(Eq. C.1)}$$

where ρ is the correlation coefficient between $\boldsymbol{\alpha}$ and $\boldsymbol{\beta}$, and σ_β^2 is the variance in $\boldsymbol{\beta}$. Significant effects produced by variations in $\boldsymbol{\beta}$ would result in the second (cubic) term in Eq. C.1 producing a significant correction to the dominant linear (αD) term in Eq. B.1.

Rough estimates for the parameters are $\rho = -0.5$ (38), σ = 0.2 Gy^{-1} (see Appendix B), and $\sigma_\beta = \langle\boldsymbol{\beta}\rangle/\sqrt{2}$ (using, for illustrative purposes, a distribution of $\boldsymbol{\beta}$ analogous to that used in Appendix B for $\boldsymbol{\alpha}$). We assume, as in Appendix B, that α = 0.3 Gy^{-1}, and also assume that a typical value of α/β (where $\beta = \langle\boldsymbol{\beta}\rangle$), for early responding tissues, is 10 Gy. Then, for a typical dose per fraction of 2 Gy, the ratio of the cubic term in Eq. C.1 to the dominant linear term (αD) is ~0.03. This crude estimate suggests that effects due to variations in $\boldsymbol{\beta}$ are small compared to the effects considered in the LQR model, i.e., due to average values of $\boldsymbol{\alpha}$, average values of $\boldsymbol{\beta}$, and variations in $\boldsymbol{\alpha}$.

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