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BIOLOGY CONTRIBUTION

INVESTIGATION OF HYPERSENSITIVITY TO FRACTIONATED LOW-DOSE RADIATION EXPOSURE

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Purpose: Hypersensitivity to cell killing of exponentially growing cells exposed to X-rays and γ rays has been reported for doses below about 0.5 Gy. The reported results have been interpreted to suggest that a dose of 0.5 Gy or less is not sufficient to trigger an inducible repair mechanism. The purpose of this study was to examine this suggested hypersensitivity after multiple low doses (0.3 Gy) of γ rays where a) the effect would be expected to be significantly magnified, and b) the effect might be of clinical relevance.

Methods and Materials: C3H 10T½ mouse embryo cells were grown to confluence in culture vessels. While in plateau phase of growth, cells were exposed to 6 Gy of γ rays, delivered in either 6 Gy, 3 Gy, 2 Gy, 1 Gy, or 0.3 Gy well-separated fractions. Corresponding experiments were performed with V-79 and C3H 10T½ cells in exponential growth. Cells were replated at low density and assayed for clonogenicity.

Results: The results of this study were not inconsistent with some hypersensitivity at low doses, in that 20 fractions each of 0.3 Gy produced a slightly lower (though nonsignificant) surviving fraction compared with the same dose given in 2-Gy fractions. However, the results of the 20 × 0.3 Gy exposures also agreed well with the standard linear-quadratic (LQ) model predictions based on high dose per fraction (1–6 Gy) data. In addition, effects of cellular redistribution were seen which were explained quantitatively with an extended version of the LQ model.

Conclusions: These experiments were specifically designed to magnify and probe possible clinical implications of proposed "low-dose hypersensitivity" effects, in which significant deviations at low doses from the LQ model have been suggested. In fact, the results at low doses per fraction were consistent with LQ predictions based on higher dose per fraction data. This finding is in agreement with the well-documented utility of the LQ approach in estimating isoeffect doses for alternative fractionation schemes, and for brachytherapy. © **1999 Elsevier Science Inc.**

Low-dose hypersensitivity, Linear-quadratic model, Dose-fractionation, Cell survival.

INTRODUCTION

The linear-quadratic (LQ) model in its standard form, or with various extensions, is used extensively in a clinical context, both to design alternative fractionation/brachytherapy schemes (1, 2), and to plan corrections for treatment interruptions (3, 4).

The mechanistic basis for the LQ model has been extensively discussed (5–7), as well as its ability to describe pertinent clinical and laboratory data (8). Recently, Joiner and colleagues have suggested that, for acute doses less than ~1 Gy, measured cellular survival in some biological systems is lower than predicted based on extrapolation—based on the LQ formalism—of data generated at higher doses (9–12). This proposed effect has come to be known as "low-dose hypersensitivity" (10, 13–18).

Clinically, if this effect were real and present in irradiated cells, it might be of some considerable significance (17, 18); for example, critical normal tissues often do receive doses in the range of 0.5 Gy per fraction, and an attempt to use the LQ model to calculate isoeffect doses, between different fractionation schemes or between fractionated and brachytherapy regimes, might lead to misleading predictions. Additionally, if resistant tumors and normal tissues showed different levels of "low-dose hypersensitivity," this difference could potentially be exploited to yield a therapeutic advantage.

Of course, there are always uncertainties in accurately measuring clonogenic survival at low doses, below ~1 Gy, in cells cultured *in vitro*, largely because of uncertainties introduced during the dilution techniques involved in stan-

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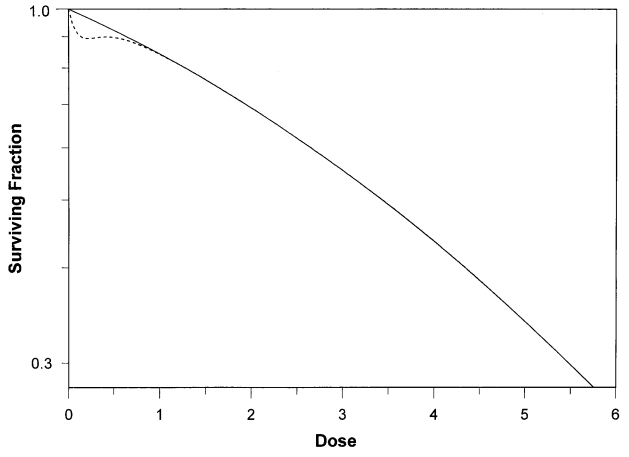

Fig. 1. Schematic of the low-dose hypersensitivity effect proposed by Joiner and colleagues (9–12). At low doses, it is suggested that the surviving fraction is lower than that predicted (solid line) by the LQ formalism parameters estimated higher doses.

dard techniques for estimating clonogenic survival (19). To overcome this problem, several methods have been employed. Some groups have made efforts to determine more precisely the number of cells plated (20) whereas others have attempted to select specific cells to evaluate after they have been plated (21). Joiner and colleagues (9–15) have used a dynamic microscopic image processing scanner (DMIPS) which allows individual cells to be located, identified and followed, to determine if they are capable of forming viable colonies. In experiments using several cell lines including Chinese hamster V-79 as well as several lines derived from radioresistant tumors, they have presented extensive data generated with the DMIPS technique suggesting that there is enhanced sensitivity to low doses of radiation, below 1 Gy (9–15). Figure 1 illustrates schematically this low-dose hypersensitivity: specifically, it is hypothesized that while cells respond in accordance with the LQ model for doses above about 1 Gy, at lower doses cells are more sensitive than the LQ predictions.

While any fine structure in the initial region of the cell survival curve is of interest in itself, to be of relevance in the clinic this low-dose hypersensitivity must be present and repeated in each of the fractions of a multifraction treatment regimen. Here we compare the effects of a fractionated low dose (6 Gy in twenty 0.3-Gy fractions) with the effects of the same dose given in larger fractions (1, 2, 3, and 6 Gy). If the effects of acute low doses (<1 Gy) are indeed larger than predicted by the LQ model, use of many low-dose fractions will magnify this difference, and the surviving fraction from the 20×0.3 Gy irradiation would be significantly lower than predicted from the LQ model using parameters derived from results obtained with higher doses per fraction.

METHODS AND MATERIALS

The cell lines used in this experiment were V-79 Chinese hamster and C3H 10T½ mouse cells. Both cell lines have been used for many years at the Center for Radiological Research, College of Physicians and Surgeons of Columbia University. The V-79 cells were grown in minimum essential medium supplemented with 10% fetal bovine serum; C3H 10T½ cells were grown in Eagle's basal medium and supplemented with 10% iron-enriched calf serum. The cells were incubated in a 37°C humidified cell culture incubator with 95% air and 5% CO_2. Both cell lines were irradiated while the cells were growing exponentially.

C3H 10T½ cells were also used in plateau phase, since they are contact inhibited and largely stop dividing when they reach confluence (V-79 cells cannot practically be kept in plateau phase over the $>$ 57 h of the 30 fraction experiment). Use of plateau-phase cells potentially represents a useful model for studying the effects of fractionation on low-dose hypersensitivity, as the complications of cellular reassortment and repopulation are much less important than with cycling cells.

For cells in exponential growth, cells were plated 6 hr before the beginning of the irradiations. For exposure of cells in plateau phase, cells were plated onto culture dishes 6 days before an experiment, so that they reached confluence 2 days before the experiment. Four dishes per run were placed in a chamber designed to provide an environment controlled for temperature, humidity, and CO_2 levels. This chamber was placed into a ^{137}Cs irradiator, which produced a dose rate of 1 Gy/min. The temporal irradiation pattern of the cells was controlled by a timer which set the exposure and interval times.

In all cases, a total dose of 6 Gy was delivered. The numbers of fractions were 20, 6, 3, 2, and 1. The time between fractions was always 3 hr, and the various exposure times per fraction were respectively 0.3 min, 1 min, 2 min, 3 min, and 6 min, corresponding to 0.3-Gy, 1-Gy, 2-Gy, 3-Gy, and 6-Gy exposures for each fraction.

At the end of the exposures, cells were trypsinized and removed from the irradiation dishes and plated into culture dishes for colony growth. Cells were incubated for 14 days, in order for growth into visible colonies and to meet the criteria of clonogenic viability. Cells were fixed with formaldehyde and stained with Giemsa before assaying for colony formation.

Four repeat experiments were carried out with exponentially growing V-79 cells, and three repeats were performed with the exponentially growing and with the plateau phase C3H 10T½ cells.

RESULTS

Results are shown in Table 1 and Figs. 2 and 3 for C3H 10T½ cells in both plateau and exponential phase, and for Chinese hamster V-79 cells in exponential growth. The results from the two different cell lines were essentially indistinguishable.

Figure 2 also shows a fit of the plateau-phase data for 1, 2, 3, and 6 fractions, *but not the 20 fraction data*, to the

Table 1. Surviving fraction (plating efficiency) for cells irradiated to 6 Gy with different numbers of well-separated fractions

Treatment	(C3H 10T½ cells (plateau phase)	C3H 10T½ cells (exponential phase)	V-79 cells (exponential phase)
Controls	(0.37)	(0.34)	(0.59)
0.3 Gy × 20 fractions	0.30	0.24	0.28
1 Gy × 6 fractions	0.36	0.33	0.34
2 Gy × 3 fractions	0.52	0.55	0.65
3 Gy × 2 fractions	0.11	0.20	0.14
6 Gy × 1 fraction	0.06	0.10	0.08

standard LQ model (1) which relates survival (S) to dose (D) as

$$S(D) = exp\,(-\alpha D - G_S \beta D^2), \qquad (1)$$

where G_S is the Lea-Catcheside dose reduction factor describing sublethal damage repair during prolonged exposure. α and β are constants. For n well-separated fractions (i.e., with times between fractions larger than typical sublethal damage repair times of 0.25 to 1.5 hr,

$$G \approx 1/n, \quad \Rightarrow \quad S(D) = exp\,(-\alpha D - \beta D^2/n), \qquad (2)$$

and it is a fit to Eq. 2 which is shown in Fig. 2. The essential point here is that the results for 0.3 Gy / fraction (20 fractions) agree well with the LQ predictions based on larger doses per fraction (1–6 Gy).

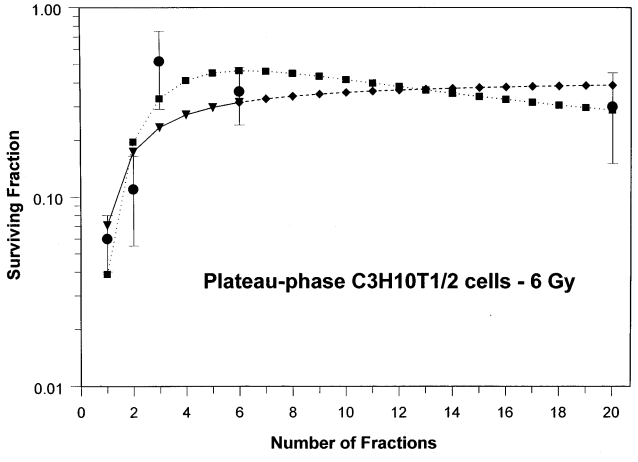

Fig. 2. Measured clonogenic survival fractions in plateau-phase C3H 10T½ cells exposed to 6 Gy of γ rays delivered in 1, 2, 3, 6, and 20 well-separated fractions. 95% confidence intervals are shown. The squares represent a fit to the model of Eqs. 3–4, which describes some low-dose hypersensitivity. The triangles represent a standard LQ model (Eq. 2) fit to the 1, 2, 3, and 6 fraction data only (i.e., not the 20 × 0.3 Gy data), and the diamonds represent the extrapolation of this standard LQ fit from ≤ 6 fractions up to 20 fractions, i.e., from ≥ 1 Gy / fraction to 0.3 Gy / fraction. Lines are shown to guide the eye only.

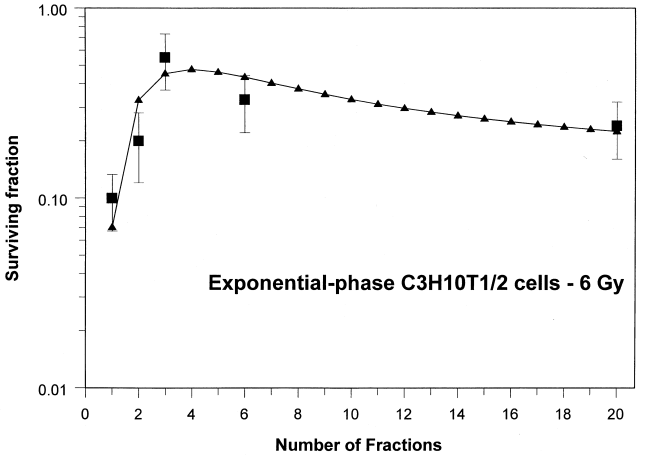

Fig. 3. Measured clonogenic survival fractions in exponentially growing C3H 10T½ cells exposed to 6 Gy of γ rays delivered in 1, 2, 3, 6, and 20 well-separated fractions. 95% confidence intervals are shown. The triangles represent an extended LQ model (LQR, Eqs. 5 and 6) fit to the data, where the model takes into account the effects of cellular redistribution between fractions. Lines are shown to guide the eye only.

Figure 2 also shows a fit of the data to an extension of the model described by Marples and Joiner (9) to describe the effect of low-dose hypersensitivity. Here, similarly to Eq. 2

$$S(D) = exp\,(-\alpha_0 D - \beta D^2/n), \qquad (3)$$

where, however,

$$\alpha_0 = \left[1 + \left(\frac{\alpha_{sen}}{\alpha_{res}} - 1\right) exp(-D/nD_c)\right]. \qquad (4)$$

Here α_{sen}, α_{res}, and D_c, as well as β, are empirically determined constants. Essentially Eq. 4 describes a situation where the linear component of the dose–response curve (α_0) increases with decreasing dose per fraction (D/n), at doses per fraction of the order of D_c.

Statistically, as the standard LQ model of Eq. 2 is a special case of Eqs. 3 and 4, it is possible to compare the residual sums of squares in either case, to test whether the standard two-parameter LQ model of Eq. 2 can be rejected, relative to the four-parameter model of Eqs. 3–4. In fact, using the appropriate F test (22), one cannot reject the standard LQ model of Eq. 2.

While the purpose of this study was to compare the results for $n = 20$ (0.3 Gy/fraction) with the LQ predictions based on the larger doses per fraction, it may be noted from Fig. 2 that the results for 3 fractions (at 2 Gy per fraction) seem somewhat higher than predicted by the models discussed here. The results from the exponential-phase experiments (Fig. 3) can shed some light on this effect at three fractions (2 Gy per fraction). Because cells are cycling, the effect of cellular redistribution (23) would be expected to be manifest as the total irradiation time increases with increas-

ing numbers of 3-hourly fractions. Essentially redistribution effects occur as cells are killed in an early fraction, and surviving resistant cells subsequently progress to a more radiation-sensitive part of the cell cycle in time for subsequent irradiation.

Figure 3 shows the result of a fit to the LQ model which was extended (24) to account for redistribution; in the extended model, designated LQR, survival is written as a function of dose D as

$$S(D) = exp\,(-\alpha D - G_S \beta D^2 + G_R \rho D^2), \qquad (5)$$

where, in the last term which describes redistribution, G_R is the corresponding Lea-Catcheside factor for redistribution effects. α, β, and ρ are constants [in the original paper (25), ρ was written as $\frac{1}{2}\sigma^2$]. Because the characteristic time for redistribution effects is likely to be much longer than that for sublethal damage repair (and longer than the 3-hr interfraction interval in the current experiments) the approximation in Eq. 2 cannot be used for G_R, which can rather be written (25)

$$G_R = \frac{2}{n^2}\left[\frac{n\theta - n\theta^2 - \theta + \theta^{n+1}}{(1-\theta)^2}\right], \quad \theta = exp\,(-T/t_R) \qquad (6)$$

where T is the time between fractions (3 hr), and t_R is a characteristic time for redistribution.

The fit of the data for C3H10T½ cells in exponential growth to Eqs. 5–6 is shown in Fig. 3. The data are highly consistent with the extended LQR model, both in terms of the point at 2 Gy per fraction (which was somewhat higher than predicted in the plateau phase results), and also in terms of the relationship between the results for $n = 20$ (0.3 Gy/fraction) and the LQ predictions based on the larger doses per fraction.

The results and fit shown in Fig. 3 for the exponential-phase data suggest that the point at $n = 3$ (2 Gy / fraction) in the plateau phase data (Fig. 2) is the result of a small amount of redistribution, i.e., the effects from a small proportion of cells in the plateau-phase population that were cycling. That plateau-phase cells do contain a significant fraction of cycling cells is well documented (26).

CONCLUSIONS

These studies extend the series of studies performed by Joiner and colleagues (9–15), who studied cellular survival in exponentially growing cells exposed to single doses of X-rays, and proposed a hypersensitivity to low doses (<1 Gy) of X-rays. Our studies were designed to use fractionation to amplify the effects of any possible hypersensitivity of cells resulting from exposure to single low-dose (0.3-Gy) fractions. In addition, the low-dose hypersensitivity phenomenon could only be of potential clinical relevance if any such hypersensitivity were maintained for each of a large number of well-separated fractions.

The results of this study were not inconsistent with some hypersensitivity at low doses, in that 20 fractions each of 0.3 Gy produced a slightly lower (though nonsignificant) surviving fraction compared with the same dose given in 2-Gy fractions. However, the results of the 20 × 0.3 Gy exposures agreed well with the standard LQ model predictions based on high dose per fraction (1–6 Gy) data. Thus there is no evidence from these experiments of any effects below 1 Gy (specifically at 0.3 Gy) which are inconsistent with the standard LQ model. This finding is in agreement with the well-documented utility of the LQ approach in estimating isoeffect doses for alternative fractionation schemes and for brachytherapy (1, 2).

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