Evidence for Two Forms of Substructure in the Cell Survival Curve

Mechanisms and Clinical Consequences

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Recent laboratory studies have clearly demonstrated the presence of two types of fine structure in the radiation survival response of cultured mammalian cells: a) one type of substructure, observed at doses of a few Gy, is the result of the differential killing of subpopulations of cells of different, cell-cycle-related radiosensitivity; this substructure is strongly dependent on the cell-cycle distribution and is absent in tightly synchronized cell populations; b) the other type of substructure, found at lower doses (< 1 Gy), is expressed as a very sensitive (hypersensitive) response at very low doses followed by increased resistance as the dose increases until, by ~ 1 Gy, the response usually follows a standard linear-quadratic (LQ) function; it thus has the characteristics of a radiation-induced radioresistance and is assumed to reflect an inducible repair process. Although the linear-quadratic (LQ) model is widely used to describe the dose-effect response both in the laboratory and in the clinic, over the past 20 years there have been several reports of an anomalous departure from the simple LQ formalism, particularly at low doses. A review of these reports suggests that the observed anomalies are not so much a failure of the LQ formalism as a manifestation of the effects of the response substructure: mixed populations, a) and hypersensitivity, b) described above.

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Since it was first described in the 1950s by Puck and colleagues (1), the mammalian cell survival curve has become the most widely used assay for examining the effects of ionizing radiation on animal systems, particularly in studies related to new strategies for radiation oncology. Although many different mathematical models have been developed over the years to describe the dose-effect response, the basic colony-forming assay used for the measurement of cell survival has changed very little over that time; it has always relied on approximately known cell concentrations and pipetting volumes in the cell suspensions which are irradiated, diluted and plated. One of the shortcomings of the classical colony-forming assay is its lack of precision at low doses (less than a few Gy), where the effects on survival are modest. Yet this is the dose range that is the most critical in radiation oncology where daily tumour doses of 2–3 Gy are commonly used. Imprecision in the measured survival at such doses can lead to considerable uncertainties in the calculated (fitted) values of the parameters associated with the various survival models such as α, β in the linear-quadratic model, for example.

In order to obtain more accurate measurements of cell survival after low doses of radiation, we have used the cell sorter assay, in which a cell sorter is used accurately to count out the number of cells plated for colony formation. This method, combined with data averaging, allows measurements of cell survival with superior precision, and is especially useful at low doses (2). Our measurements have revealed that there is substructure in the radiation response of asynchronously dividing cells. One type of substructure, observed at doses of a few Gy, has the characteristics of a 2-component response when the LQ model is fitted to the data. This is consistent with the presence of subpopulations of cells of different radiosensitivity, associated with different stages of the cell mitotic cycle. The absence of such substructure in the radiation response of homogeneous, tightly synchronized cell populations lends strong support to the subpopulation explanation for this substructure (3–5). We have applied the cell sorter assay in order to examine the radiation response of a number of Chinese hamster and human tumour cell lines, in both asynchronous and synchronous populations. The cell lines studied include V79-171 (2–4) and V79-WNRE (5, 6)

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hamster lines and HT-144 (melanoma), MCF7 (breast), SiHa (cervical Ca), U-373 MG (glioblastoma), A549 (lung adenocarcinoma), HT-29 (colon adenocarcinoma), DU 145 (prostate Ca) and U1 (melanoma) human tumour cells (3, 7, 8). Asynchronous cultures of the rodent cell lines and most of the human tumour cell lines exhibited the 'mixed population substructure'; where homogeneous synchronized populations were obtained, most of the cell lines yielded radiation responses which were well described by a single LQ function, lending support to the validity of this model of cell inactivation. One exception was U1 cells, which seem to have an anomalous response (7).

We have utilized the cell sorter assay to investigate a number of practical problems, including a) a study of radiosensitizer mechanisms—we have shown that etanidazole and nimorazole enhance primarily the β term (2 hit) of tumour cell inactivation (9), while RB 6145 enhances primarily the α term (1 hit) (8); this suggests that sensitizer efficacy may be tumour-specific and predictable from tumour response parameters. b) measurement of the relative biological effectiveness (RBE) in a 70 MeV clinical proton beam—RBE was found to be dependent on depth and on dose, the latter being strongly influenced by population substructure in the radiation response of the cells used (10). Log phase and lag phase cultures of the same cell line (V79-WNRE) show very different dependence of RBE on dose.

The cell sorter assay has also been used to investigate a second order of substructure, termed the hypersensitive response, first described by Marples & Joiner (11). This substructure is observed at significantly lower doses (< 1 Gy) than the population substructure discussed above. Its features are consistent with a dose-dependent induction of repair rather than a sub-population of extremely radiosensitive cells.

The discussion section of this report will examine the possible involvement of these two forms of response substructure in a number of reports of anomalous departures from a simple LQ dose-effect relation, observed in in vivo studies during the past 15 or 20 years.

MATERIAL AND METHODS

Cells

Chinese hamster V79-WNRE cells (a subline of V79 379A) were routinely maintained in suspension culture in calcium-free minimum essential medium (SMEM, Gibco, Burlington, ON) supplemented with 7% foetal bovine serum (FBS), sodium bicarbonate and antibiotics as described previously (5). Prior to the experiments, the cells were transferred to monolayer culture (MEM + 12.5% FBS) and grown to 50–70% confluency. Asynchronous cell populations were harvested from these cultures by trypsinization, providing single-cell suspensions free of aggregates. Cells were irradiated (250 kVp x-rays, HVL 1.5 mm Cu) in suspension in water-jacketed (37°C) spinner flasks gassed with humidified air.

Synchronized V79-WNRE cell populations were obtained through a combination of mitotic selection and metabolic block to arrest cells at the G_1/S border (5). A Talandic cell cycle analyser (Talandic Research Corp., Pasadena, CA) was used for mitotic selection. The cells were grown as monolayers in large (1750 cm^2) roller bottles, rotating at 1 rpm in a 37°C warm room. Harvest of mostly mitotic cells was accomplished using a high rotation speed (200 rpm) which dislodged these loosely attached cells. The cells were then transferred to MEM medium containing 1mM hydroxyurea in monolayer culture flasks, and incubated for 12 h to accumulate the cells at the G_1/S border. These tightly synchronized cell populations were then trypsinized and irradiated in suspension, as above. The purity of the synchronized cell populations was monitored using mitotic index analyses and flow cytometry (5).

The human tumour cells (HT-29: colon adenocarcinoma) were obtained from American Type Culture Collection. They were cultured as monolayers in McCoy's 5A medium supplemented with 10% FBS, sodium bicarbonate and antibiotics, as described previously (7). Asynchronous cell populations were harvested for experiments by trypsinization of the monolayer cultures. The experiments with HT-29 cells that are reported here used a special irradiation and plating procedure described below (hypersensitive response).

Cell sorter assay

Cell survival was determined using a cell sorter (Becton-Dickinson 440) to sort an accurately known number of cells from each sample. Single cells were identified on the basis of forward and perpendicular light scatter without the use of a cell stain (12). Three sorts were dispensed from each sample (two samples per dose point) into test tubes containing 4.5 ml growth medium. Each sort was then plated into a 100 mm Petri dish with an additional 10 ml warm growth medium containing (for human cells only) 70000 heavily irradiated (70 Gy) feeder cells. After 7 or 14 days' growth (for V79 or HT-29, respectively), colonies were stained and counted manually.

Hypersensitive response

In order to obtain higher precision in our measurements of HT-29 cell survival at a very low dose (< 1 Gy), we used a refinement of the cell sorter assay in which the cells were sorted prior to irradiation (13). After sorting, tubes (6 for each dose point) containing known numbers of cells were irradiated at 37°C and plated within a consistent 6–8 min period.

Data analysis

The plating efficiency and surviving fraction were determined for each Petri dish. Data from several experiments were pooled together, and the mean survival and standard error of the mean were calculated for each dose point. Several different mathematical models were fitted to the survival data in the course of these studies. The best-fit parameter values in these models were obtained using a non-linear curve-fitting programme with appropriate weighting of survival data based on the scatter in the measured data. Further details of the statistical analysis can be found elsewhere (5, 7, 13).

RESULTS

Mixed population substructure

Fig. 1 shows the radiation survival response of asynchronously dividing V79 WNRE cells, measured using the cell sorter assay. Fig. 1a shows the usual semi-log plot of surviving fraction vs. dose, while Fig. 1b shows the same data plotted as $-(\ln S)/\text{dose}$ vs. dose. The linear quadratic (LQ) survival function

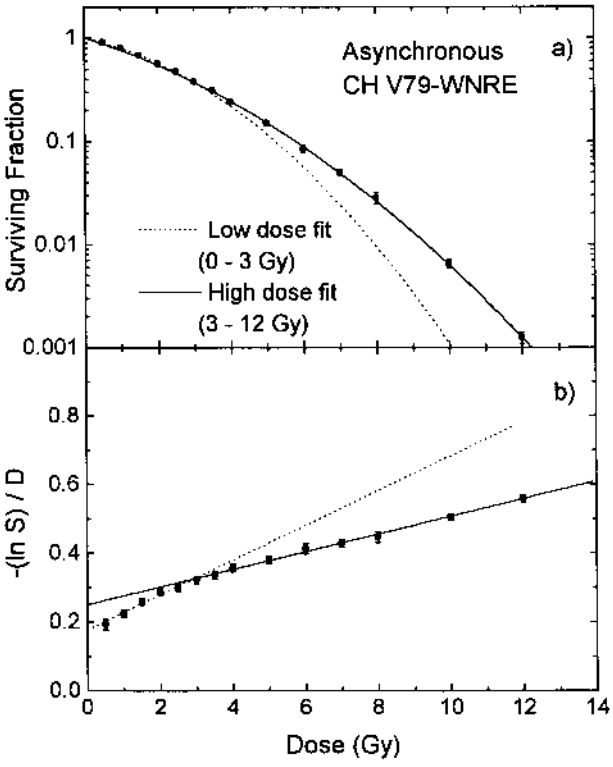

Fig. 1. Survival response of asynchronously growing Chinese hamster V79 WNRE cells, measured using the cell sorter assay. a) Plotted as S vs. Dose (equation [1]); b) the same data as in a), but plotted as $-(\ln S)/D$ vs. Dose (equation [2]). In b), the y-axis intercept is α, slope is β. Data are the average of five responses. Dashed lines: LQ fit to low dose data, 0–3 Gy; solid lines: LQ fit to high dose data, 3–12 Gy. Error bars are the standard error in the mean. (Adapted from (5)).

$$S = \exp(-\alpha D - \beta D^2) \quad [1]$$

has been used to fit the data. When the low dose (0–3 Gy) and high dose (3–12 Gy) data are fitted separately to equation [1], distinctly different responses are obtained with significantly different parameter values (5), as shown in Fig. 1a. This divergence between the low dose and high dose fits can be more clearly illustrated if we 'linearize' the LQ function as shown in Fig. 1b by taking the log of the function and dividing by dose:

$$-(\ln S)/D = \alpha + \beta D \quad [2]$$

Plotted in this form, data that are adequately characterized by a single LQ function should follow a straight line of slope β and y-axis intercept α. The clear 2-component response of Fig. 1b suggests (at least) 2 subpopulations of cells, one sensitive and the other more resistant. Since these asynchronous cell populations are known to be comprised of subpopulations of cells of different cell-cycle-dependent radiosensitivity (e.g., sensitive M-, G_1-, G_2-phase and resistant late S-phase cells) one can postulate that the substructure evident in Fig. 1 is just a reflection of this situation: the selective killing of the more sensitive cells and the emergence of a more resistant surviving population at high dose.

If this explanation for the response substructure evident in Fig. 1 is correct, then there should be no such substructure present in the response of a homogeneous population of synchronized cells, provided, of course, that the LQ function correctly describes the radiation response of a homogeneous cell population. The results of an experiment designed to test this hypothesis are described in Fig. 2, where a combination of mitotic selection and metabolic inhibition was used to produce a tightly synchronized population of cells arrested at the G_1/S border. The data here are plotted in the same fashion as those in Fig. 1. In Fig. 2, however, there is no significant substructure, i.e., the responses fitted to the low-dose and high-dose data have parameter values that are not significantly different (5). These results thus support the 'mixed population' explanation for the substructure in the survival response of Fig. 1.

Asynchronous populations of another Chinese hamster cell line, V79-171 (2–4), and of several different human tumour cell lines (7, 14) have yielded survival responses very similar to those presented in Fig. 1, which suggests that this substructure is a common, though perhaps not universal (7, 14), feature of radiation responses, at least as measured in vitro. Tightly synchronized populations of V79-171 cells (3, 4) and of HT-29 human tumour cells (14), produced homogeneous responses very similar to those in Fig. 2, offering further support for the hypothesis that a substructure of the type shown in Fig. 1 for asynchronous cells is due to subpopulations of cells of different, cell-cycle-related radiosensitivity.

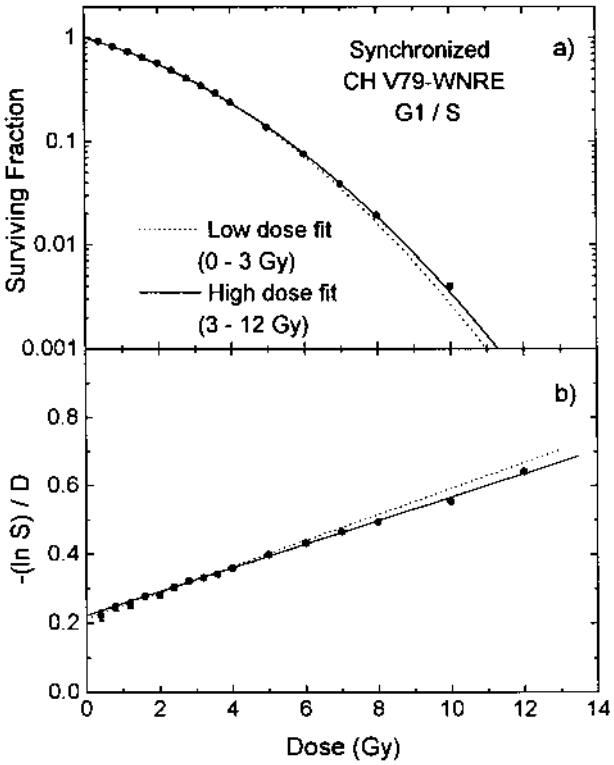

Fig. 2. Survival response of synchronized G_1/S V79 WNRE cells, measured using the cell sorter assay. a) Plotted as S vs. Dose (equation [1]); b) the same data as in a), but plotted as – (ln S)/D vs. Dose (equation [2]). Cells were synchronized by mitotic selection followed by 12-h exposure to 1mM hydroxyurea, to accumulate cells at the G_1/S border; cells were irradiated 30 min after removal of hydroxyurea. Data are the average of seven responses. Fitted lines and error bars as in Fig. 1. (Adapted from (5)).

The possible significance of this radiation response substructure in radiation oncology is examined in the following discussion.

Hypersensitive response substructure

In the work described to this point the emphasis has been on the 'clinically relevant' low-dose region of a few Gy, and in fact the lowest dose point used was 0.4 or 0.5 Gy (see Figs 1 and 2). Fig. 3 presents the results of a subsequent investigation, using the cell sorter assay, of another form of response substructure that is found at lower doses, 0–1 Gy (13, 15). The data clearly indicate a region of increased radiosensitivity (hypersensitivity) at doses < 0.5 Gy, approximately, in these asynchronous HT-29 cells. Similar responses were observed for three other human tumour cell lines: DU 145 (prostate Ca), A549 (lung adenocarcinoma) and U1 (melanoma). The results are consistent with the earlier findings of Marples & Joiner at the Gray Laboratory (11). They observed a very similar hypersensitivity in Chinese hamster V79 cells, using an automated microscopic assay (16, 17) for the measurement of cell survival, another method that provides good accuracy at low doses. Low-dose hypersensitivity was also observed in several human tumour cell lines by the Gray Laboratory group (18–20). Marples & Joiner and their colleagues interpreted the hypersensitive response and subsequent increase in radioresistance as evidence of a process of radiation-induced radioresistance (repair?). Analysis of our data led to the same conclusion and both groups developed models which, in different ways, incorporated induced radioresistance to describe the hypersensitive response (13, 15; 11, 21).

DISCUSSION

The data presented in Figs 1–3 represent examples of a substantial body of data now published which illustrates the presence of fine structure (substructure) in the radiation survival response of mammalian cells in vitro. The ability to resolve this fine structure has been greatly enhanced by the development of two techniques, the cell sorter assay and the automated microscopic assay

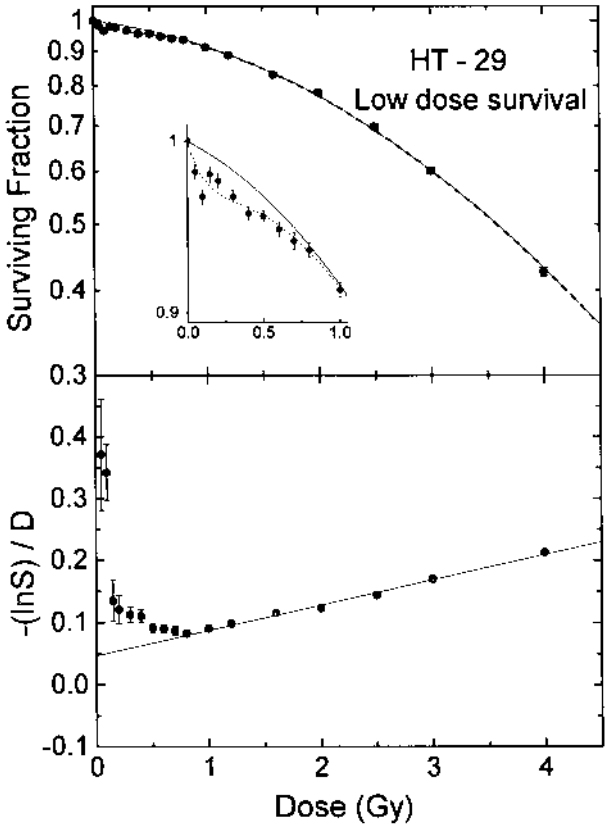

Fig. 3. Hypersensitive response of HT-29 cells, evident at doses below ~ 1 Gy, measured using the cell sorter assay. a) Plotted as S vs. Dose (equation [1]); inset shows an expansion of the 0–1 Gy region. The solid line represents the best LQ fit to the data from 1–4 Gy. The dashed line is the best fit of the whole data set to the variable – α induced radioresistance model, where α is assumed to be a function of dose (13, 21). b) The same data as in a), but plotted as – (ln S)/D vs. Dose (equation [2]). Error bars are the standard error in the mean. (Adapted from (13)).

(DMIPS), both of which offer improved precision for measurements of cell survival. The 'hyper-sensitive' substructure has been demonstrated with both techniques and in two different laboratories, while the 'mixed population' substructure is only accessible using the cell sorter assay (the microscopic assay is only practical for the first decade of cell inactivation, which is insufficient to demonstrate the mixed population substructure). When viewed in the context of a 2- or 3-decade plot of the surviving fraction vs. dose, both effects are small and for many radiobiological measurements their significance may be limited. However, the impact of this response substructure on our understanding of radiobiological mechanisms may be substantial as it emphasizes the importance of the cell's active response to radiation insult and the role of population dynamics in the response. And certainly the improved accuracy of response measurement has allowed more precise determinations of widely used parameters such as α and β (7) and of RBE (10).

It is necessary to recognize, however, that all of the studies we have described involve single-dose irradiation of exponentially growing cultured cells in vitro. Is there any reason to suppose that either of these two forms of substructure (which we shall refer to as 'mixed population' and 'hypersensitive') is manifested in a typical, multifraction radiation therapy treatment? In other words, do the results have any clinical significance?

Mixed population substructure

We have published data for 8 human tumour cell lines and 2 rodent lines which demonstrate that, in general, α, β and particularly α/β are dose-dependent (2–5, 7, 8). The value of α/β typically decreased at low dose and at 2 Gy was found to be 2–3 times smaller than at higher doses, as would be determined in a conventional measurement (7). This could have an impact, for example, on estimates of the biologically effective dose (BED) for a new treatment protocol since such projections are usually based on the LQ model and employ α/β values which have typically been measured (in experimental animals) at doses per fraction of 5 Gy or more (22–24). But to suggest that the results of our in vitro measurements may be relevant to the response of either the tumour or a dose-limiting normal tissue in such a new protocol implies a number of assumptions:

1. that the clonogenic cells of interest are cycling and continue to cycle throughout most of the treatment regime (and have a radiosensitivity which is cycle-dependent);
2. that the time interval between dose fractions is substantially greater than any radiation-induced mitotic delay; and
3. that the possible confounding effects of cell repopulation, incomplete repair, radiation-induced synchronization, etc., do not overwhelm the effects of population inhomogeneity and consequent differential cell killing.

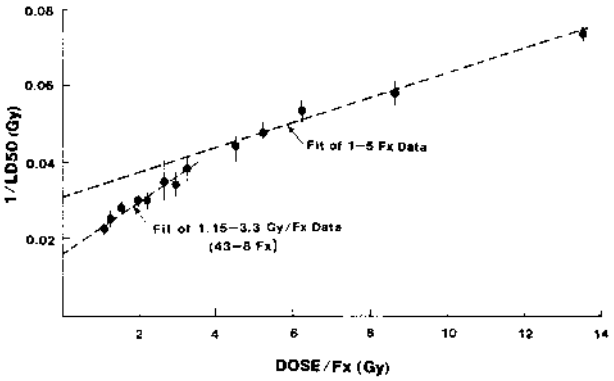

Fig. 4. F_e plot for mouse lung death (80–120 days)—reciprocal isoeffect total dose, $1/LD_{50}$ vs. Dose per fraction. The fitted values of α/β were 2.2 Gy and 10.2 Gy for the low and high dose ranges, respectively. Error bars are 95% confidence intervals. (From Vegesna et al. (25), reprinted with the permission of Taylor & Francis).

With all of these factors arguing that the mixed population substructure may not be manifested in vivo, it is perhaps surprising that there is, in fact, considerable evidence in the literature to support it. Fig. 4 presents the data of Vegesna et al. for the response of mouse lung to radiation delivered in different fractionation regimes (25). It should be noted that plotting the reciprocal of the total isoeffect dose vs. dose per fraction, as in this figure, is exactly analogous to our $-(\ln S)/D$ vs. dose plot and it has the effect of linearizing the LQ function (26). The plot of Fig. 4 shows the same characteristics as those found in Fig. 1b. The ratio of the y-axis intercept and the slope of the plot again gives α/β. The values of α/β for the high-dose and low-dose fits shown in this figure are 10.2 and 2.2, respectively. These authors concluded that the non-linearity of the reciprocal isoeffect curve of Fig. 4 was due either to the failure of the LQ model to correctly describe the dose-response relation for the target cells for lung death (perhaps at a higher dose the survival vs. dose response becomes more nearly exponential, as in the 2-component model; this would approach a horizontal line at high dose in Fig. 4) or it was due to incomplete repair during the 3-h intervals between dose fractions in their study (or a combination of these two effects). Certainly, incomplete repair can produce a non-linear reciprocal isoeffect response, similar to that found in Fig. 4, as has been shown by several studies (e.g. 27, 28); basically, the major effect of incomplete repair is at intermediate doses, the 'region of flexure', where it enhances the effectiveness (pushes the isoeffect curve upward). However, in the Travis et al. study (27), which also used mouse lung, this non-linearity was only evident for interfraction intervals of 2 h or less. The alternative explanation, that the LQ model

is inappropriate, has been offered essentially each time that a non-linear isoeffect response (or F_e plot, as it is often called) has been observed (e.g. 29). But there has been little independent or mechanistic evidence put forward to confirm this hypothesis.

An entirely different explanation for the non-linear F_e plot was subsequently offered by Schultheiss et al. (30). They modelled the reciprocal isoeffect response (F_e plot) using the LQ dose-effect relation but taking into account population heterogeneity in radioresponsiveness and, in fact, they used the V79 cell-cycle data of Kruuv & Sinclair (cell survival curves at various stages of the cell cycle) (31) as the input for their calculations. The results of their calculations are shown in Fig. 5. Curve B, which represents the response for a heterogeneous population, is very similar to Fig. 1b and 4 and shows that heterogeneity in radioresponsiveness of mixed cell populations necessarily leads to response substructure (or non-linearity) of the type that we have observed, even when homogeneous subpopulations follow a simple LQ dose-response function. More recent studies by Hopewell et al. showed a similar non-linearity in the reciprocal isoeffect curve for the early reaction in pig skin, even when correction was

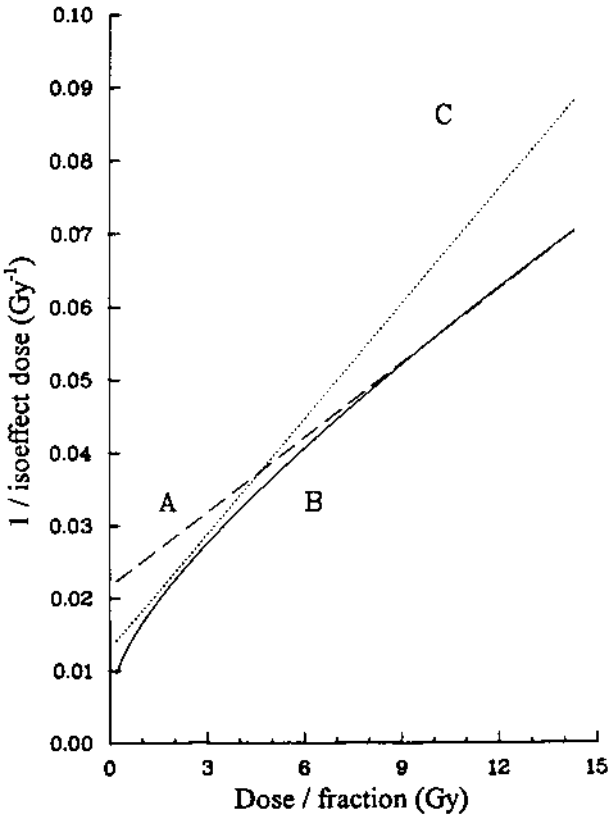

Fig. 5. Calculated F_e plot for mathematically modelled homogeneous (A, C) and heterogeneous (B) tissue cell populations. The values of α/β were 6.3 and 2.45 for A and C, respectively. (From Schultheiss et al. (30), reprinted with the permission of Elsevier Science).

made for possible incomplete repair (32). The α/β ratio at low dose per fraction was approximately 10-fold smaller than at high dose per fraction. Dermal necrosis, a late effect in pig skin (32) and late lung damage (reduced lung function) (33) also indicated a decreased α/β ratio at low dose per fraction. Studies by Wong et al. (34, 35) of the effects of small doses per fraction on rat spinal cord gave similar results: the value of α/β decreased sharply at low dose per fraction (down to 0.55 Gy/fraction). In these studies, an interfraction interval of 24 h was used to avoid the possible complication of incomplete repair. All of these in vivo results, therefore, fit well with our in vitro observations of a reduced α/β ratio at low dose, associated with mixed cell populations of heterogeneous radiosensitivity. This result is predicted by the theoretical modelling of Schultheiss et al. (30), described above.

Finally, is there any clinical evidence to suggest that this dose-dependence of α/β that we have observed and studied in vitro and that others have observed in vivo could also occur in humans receiving radiation therapy? Note that irrespective of the underlying basis of this dose-dependence of α/β which we have observed (and attributed to mixed cell populations), its consequence is that there should be greater tissue sparing available from increased fractionation (hyperfractionation) than would be predicted by extrapolation of the higher dose/fraction data, with its larger α/β value; thus, calculating a new hyperfractionated 'Effective Dose' regime based on the usual LQ formalism (23) and a high dose α/β value should underestimate the isoeffective dose for the dose-limiting tissue in question. As an example of the general magnitude of this effect, if the low-dose α/β were only one-third as large as the high-dose α/β, an Effective Dose calculation based on the high-dose α/β would underestimate the isoeffect dose by approximately 10% for a typical situation, large enough to be of clinical significance. It is interesting to note that in the randomized CHART (continuous, hyperfractionated, accelerated radiotherapy) trial for head and neck cancer in the UK (36), late effects were less severe in the CHART arm (54 Gy in 36 fractions of 1.5 Gy, 3/day with at least a 6 h interval, in 12 consecutive days) than in the conventional arm (66 Gy in 33 fractions of 2 Gy in 6.5 weeks). This occurred despite the fact that the CHART dose was calculated (using the LQ formalism) to produce equivalent late effects in the two arms. However, in this calculation the common assumption was made that the value of α/β for late effects is not dependent on dose per fraction. The tissue sparing that was observed in the CHART arm, therefore, is exactly what we would predict if, instead, the value of α/β decreased at a lower dose per fraction. At the same time, some of the early reactions, particularly mucositis, were more severe in the CHART arm. This again is to be expected, given the generally smaller slope of the F_e plot for early reactions ($\alpha/\beta \sim 10$) vs. late reactions ($\alpha/\beta \sim 3$): a hyperfractionated dose which is calculated to be

isoeffective for late effects may tend to over-treat early-reacting tissues. Even if there were, in addition, a decrease in α/β at low dose per fraction for early-reacting tissues, this might not be sufficient to offset the overall smaller slope of the F_e responses for acute effects.

Hypersensitive response substructure

This substructure is observed at lower doses than the mixed population substructure and it has the opposite effect on the radiation response. That is, whereas the presence of mixed population substructure has been observed as a less sensitive response at low doses (a few Gy) than would be predicted by a simple LQ extrapolation from higher dose data, hypersensitivity would be observed as a more sensitive response at low doses (below 0.5 to 1.0 Gy). But is hypersensitivity at low dose observed in vivo? In fact, the in vitro experiments of Marples & Joiner (11) were preceded by in vivo studies in the same laboratory in mouse skin (37) and mouse kidney (21), which showed enhanced radiosensitivity at low doses. A similar result had been previously reported by Ang et al. for irradiation of rat spinal cord (38). Yet, since the hypersensitivity is only observed at much lower doses than the usual prescribed target volume dose, is the effect of clinical significance? This raises several issues: are there clinical situations where dose-limiting normal tissues could receive doses per fraction too small to 'induce repair' and yet where the total dose will be sufficient (with hypersensitivity) to lead to a clinical complication? In fractionated treatments, only tissues outside the treatment volume could receive low enough doses (even with hyperfractionation) to express hypersensitivity; and one would need to postulate that there could be no cumulative or memory effect between dose fractions, to 'turn on' the radioresistance as the dose accumulates.

There is at least one report of a clinical study that explores the question of hypersensitivity to low dose in human skin (39). This Australian study by Hamilton et al. included two groups of patients: one group of 65 patients received palliative treatment to various tumour sites, with the dose per fraction to skin ranging from 0.4 to 5.2 Gy/fraction in protocols that utilized 5, 10, 12 or 20 fractions; a second group of 52 patients being treated for prostate cancer received a mean skin dose per fraction of 0.42 Gy in a 32 fraction protocol. They present their skin erythema results in a reciprocal dose (F_e) plot, analogous to Fig. 3b, which presents clear evidence of hypersensitivity for the 20- and 32-fraction patient groups, for which the skin dose per fraction is less than 1 Gy. The interpretation of these results has come under some discussion and debate (39–42) and it is hoped that the findings of Hamilton et al. will stimulate further clinical studies of this type, in order more completely to define the possible role of hypersensitivity or induced radioresistance in clinical radiotherapy.

The potential clinical importance of hypersensitivity has been underlined by the recent theoretical analyses of Dasu & Denekamp (43), which incorporate the concept of inducible repair in the radiation response of aerobic and hypoxic tumour cells. Their results show that hypersensitivity at low dose can have a dramatic effect on hypoxic protection and cell kill and that the manner in which the dose is delivered can substantially alter these effects.

CONCLUSIONS

During the past two decades or so, there have been several reports of detailed studies, both in vitro and in vivo, where, although the linear quadratic model provided a good general description of the dose-effect response, there were situations where significant departures from the simple LQ formalism were evident, particularly at low doses. Although factors such as repair and repopulation could complicate the in vivo studies, it is very likely that the response substructure described in this paper is at least part of the 'departures' from the LQ formalism mentioned in these reports. Recognition of their potential importance and a better understanding of the impact of these two types of response substructure should allow more compete descriptions of radiation response and hence more precise prediction of effects in the development of new treatment strategies.

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