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Parameters of linear-quadratic radiation dose-effect relationships: dependence on LET and mechanisms of reproductive cell death

G. W. BARENDSEN

Abstract. An analysis of mammalian cell radiation–dose survival curves, based on the linear–quadratic formalism, is shown to yield insights in the various components of damage that contribute to cell reproductive death. *RBE*–LET relationships of single-track lethal damage, sublethal damage, potentially lethal damage and DNA double-strand breaks are compared. Single-track lethal damage is derived to be composed of two components: (1) damage that remains unrepaired in an interval between irradiation and assay for proliferative capacity, with a very strong dependence on LET, and (2) potentially lethal damage that is only weakly dependent on LET, similar to sublethal damage and DNA double-strand breaks. The results of this analysis lead to new interpretations of published experimental results and to suggestions for applications in radiotherapy.

1. Introduction

Ever since the first results obtained by the cloning technique of Puck and Marcus (1956) were published, differences in shapes of mammalian cell survival curves have been discussed by many investigators, among them Tikvah Alper quite prominently (Alper *et al.* 1960). Interest in this subject derived not only from its relevance to the understanding of mechanisms of biological radiation damage, but also from the implications for dose–effect relations in radiotherapy of malignant diseases. Normal tissue damage as well as tumour control depends on radiation responses of constituent cells, in particular impairment of their capacity for unlimited proliferation. Of special importance is the induction of reproductive death by mechanisms that yield lethal lesions as a linearly increasing function of the dose and independent of the dose-rate. These mechanisms determine to a large extent the effectiveness at small doses of 0–2 Gy, while the contribution of damage due to accumulation and interaction of sublethal lesions starts to dominate at larger doses (Barendsen 1962, 1979, 1982). Insights with respect to mechanisms of induction and repair of various types of damage involved in responses of mammalian cells and tissues contribute to a rational basis for the selection of optimal doses and treatment schedules to achieve tumour control without unacceptable normal tissue damage (Fowler 1989).

In studies concerning mechanisms that determine radiosensitivities of mammalian cells, three different approaches can be distinguished which complement each other and together eventually should result in a complete description of the processes of induction and expression of cellular damage (Barendsen 1990).

In the biophysical approach dose–effect relations are studied with respect to their dependence on two major physical factors: (1) the distribution of dose in time and (2) the spatial distribution of energy deposition along the tracks of ionizing particles in cells. Studies of the influence of dose fractionation and dose-rate have shown the importance of the contribution of sublethal damage (SLD) to cell lethality and of the time interval in which repair of this damage can be completed (Elkind and Sutton 1960, Elkind and Whitmore 1967, Fowler 1989). Accumulation and interaction of sublethal damage yields the increase in slope of survival curves with increasing doses of radiation of low linear energy transfer (LET) (Elkind and Whitmore 1967). In addition to lethal and sublethal lesions, the induction and expression of potentially lethal damage (PLD) has been established and repair of PLD has been assessed as a function of the time interval after irradiation (Phillips and Tolmach 1966, Iliakis 1988).

Studies of the influence of energy deposition density along tracks of ionizing particles and in volumes of subcellular dimensions, using different ionizing particles, have shown that local clustering of damage is a major factor in the induction of cell reproductive death and chromosomal damage in mammalian cells (Barendsen *et al.* 1960, 1963, Barendsen 1967, 1979). In particular, lethal lesions that increase linearly with dose are more efficiently produced as the LET increases, reaching a maximum in the region of 100–200 keV/μm. Sublethal damage is much less dependent on LET (Barendsen 1993). Microdosimetric information is of major importance for the interpretation of these differences in dependence of the relative biological effectiveness (*RBE*) on LET (Barendsen 1962, 1964, 1979, Kellerer and Rossi 1972, Goodhead 1989). In the past two decades more detailed information has become available on the track structures of ionizing particles. The influence

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of LET on cellular effects can now be related to the effectiveness for the induction of DNA damage, e.g. single-strand breaks (ssb), double-strand breaks (dsb) and base damage, with the aim of evaluating which targets and mechanisms are critical in the initiation of cellular damage. Charlton *et al.* (1989) calculated on the basis of this type of information that the induction of DNA dsb increases with the amount of energy between 100 and 300 eV deposited locally in DNA, but that the *RBE* does not attain values as high as obtained for cell inactivation. This result is in agreement with experimental data on the LET dependence of DNA dsb.

In the biochemical approach studies are directed at assessment of the chemical processes which cause changes in various constituents of cells, in particular in DNA. These studies involve evaluation of the contributions of water radicals, of the influence of sensitizing and protecting compounds, e.g. molecular oxygen, SH-compounds, alkylating agents, and of other cytotoxic agents. Alper is well known for her contribution to this type of studies (Alper and Bryant 1974). Ward (1990) has concluded from a review of the yields of damaged moieties in intracellular DNA that OH radicals are responsible for about 60% of the strand breaks. He has suggested that lethal lesions result from locally multiply damaged sites (LMDS) in DNA. If the damages in these LMDS are within a few base pairs of each other, loss of base sequence information can occur during repair by various pathways.

An important deduction from studies of radiation-induced effects in DNA is that in mammalian cells the number of DNA ssb is approximately 1000 times larger and the number of DNA dsb is approximately 50 times larger than the number of lethal events and chromosome aberrations induced by a given dose. This information has led to the insight that in particular mammalian cells have a very large capacity for repair of radiation-induced damage. It is well known that, related to the amount of DNA present in cells, mammalian cells are more resistant than yeast, bacteria or viruses, suggesting that lethal events in DNA are less efficiently induced in mammalian cells (Terzi 1961, Kaplan and Moses 1964). This can be interpreted to be due to a stronger clustering of damage in DNA being required to cause reproductive death in mammalian cells as compared to other types of cells. This hypothesis is consistent with the observation that for mammalian cell reproductive death the strongest increase of *RBE* as a function of LET is obtained, a consequence of the inefficient production of strongly clustered damage by low-LET radiations. Studies on the modification of DNA structure, e.g. by the uptake of halogenated pyrimidines which

increases radiosensitivity, can provide further insight in the type of changes causing cellular damage (Szybalski 1974, Miller *et al.* 1992, Jones *et al.* 1995).

The third approach, which in particular in the last decennium has contributed to developments in radiobiology, is based on molecular biology methods. Many studies using these methods are now directed at the elucidation of cytogenetic mechanisms of repair of DNA damage, of control of cell cycle progression and of carcinogenesis. A number of genes involved in DNA repair has been identified and their function characterized (Lohman *et al.* 1995). It has been suggested that tumour cells may differ with respect to their repair proficiency and that this factor may affect the response to therapy (Weischelbaum *et al.* 1984). However, comparison of cell lines with different radiosensitivities has not revealed consistent differences in repair rates. Chromosome aberrations can now be detected and analysed in more detail using the technique of chromosome painting. It has been shown that ionizing radiations cause effects mainly by induction of deletions rather than by point mutations (Rydberg 1996).

All three types of approaches mentioned contribute to the development of models designed to describe the complete sequences of changes induced by ionizing radiations, starting with energy deposition in critical structures or molecules in cells, enhancement by the clustering of damage, modification by repair mechanisms and finally resulting in reproductive death, or carcinogenesis. To be useful in radiotherapy and radiation protection, the results of these descriptions must be expressed in terms of parameters that provide quantitative data on probabilities for the endpoints considered, in particular at low doses relevant to these applications. In the following sections some insights concerning the influence of clustering of damage, mainly derived by the biophysical approach, will be discussed and implications for radiotherapy will be briefly indicated.

2. Survival curves and their dependence on LET

The first radiation dose-survival curve of mammalian cells in culture, published by Puck and Marcus (1956), was interpreted by assuming that two targets had to be inactivated to cause cell reproductive death. A multitarget mechanism results in a survival curve characterized by an initial low-dose region starting with a zero slope, followed beyond a shoulder region by an exponential decrease of the surviving fraction at larger doses in excess of about 5–10 Gy. On a semilogarithmic plot extrapolation of the exponential region to dose zero yields the extrapolation

number (Alper *et al.* 1960). This number was soon observed to vary greatly for different cell types and with various conditions of culture. The multitarget model had to be adapted to accommodate this variation and as a consequence the biophysical significance of the extrapolation number remained ambiguous (Elkind and Whitmore 1967).

The initial suggestion that survival curves of mammalian cells for low-LET radiations cannot be adequately described by multitarget models, but that a component with linear dependence on the dose is required to fit the low-dose region of the curves, was based on a comparison of survival curves measured for low- and high-LET radiations (Barendsen *et al.* 1960, 1963, Barendsen 1962). The first survival curve of mammalian cells, irradiated *in vitro* with alpha-particles at a high LET of 140 keV/ μ m showed an exponential decrease of the surviving fraction with the dose:

$$s(D)/s(0) = \exp - a_1 D.$$

This result was interpreted by the assumption that cell reproductive death can be induced by the passage of a single particle through a critical structure or molecule of the cell (Barendsen 1962). This type of lethal event is denoted single-track lethal damage (STLD). Subsequent studies with alpha particles and deuterons covering a wide range of LET between 10 and 200 keV/ μ m showed a decreasing contribution of the linear component with decreasing LET (Barendsen *et al.* 1963, Barendsen 1967). However, even at 10–20 keV/ μ m a significant linear component could be determined, causing an initial negative slope of the survival curves at low doses. This result suggested that also with other radiations of low LET, e.g. electrons generated by photons, an important contribution of a linear component should be present. This component is due to damage induced by slow electrons, which have an increased LET at the end of their tracks. This was later verified by many studies with small doses per fraction and with doses delivered at low dose-rate (Barendsen 1962, 1979, Hall 1972).

To describe mammalian cell survival curves that show on a semilogarithmic plot an increase in slope with increasing dose, the most simple equation is obtained by adding in the exponent a term quadratic in the dose:

$$s(D)/s(0) = \exp -(a_1 D + a_2 D^2).$$

†This representation provides a generally adequate

description of the shapes of survival curves up to doses of about 10 Gy. It describes a curve which continues to increase in slope with increasing dose (Barendsen 1962, 1979, Kellerer and Rossi 1972).

For survival curves which at large doses show an exponential decrease in survival, another representation, including a term similar to the multitarget formula, can provide a satisfactory fit to the data. This formula requires two parameters, D_n and n , in addition to the parameter describing the linear component:

$$s(D)/s(0) = (\exp - a_1 D)(1 - (1 - \exp - D/D_n)^n).$$

The following discussion will be confined to the region of surviving fractions between 1 and 0.01 for most cell types corresponding to doses or doses per fraction in the range of 0–5 Gy for which the representation by the linear-quadratic formula is adequate.

3. LET dependence of STLD

The component of lethal lesions that increases linearly with the dose is generally observed to show a strong increase of the *RBE* with LET between 10 and 100 keV/ μ m, to maximum values in the range 5–10, with a subsequent decrease at LET > 200 keV/ μ m (Barendsen 1979, 1990). An example of this relationship is given in Figure 1. The *RBE* represent the ratios a_{1H}/a_{1L} , in which these linear parameters a_{1H} and a_{1L} represent the effectiveness of high- and low-LET radiations at low doses respectively. The two different *RBE* curves in Figure 1 pertain to oxygenated and hypoxic conditions. The larger *RBE* in hypoxic conditions are due to the fact that the oxygen enhancement ratio (*OER*) is larger for low-LET radiation than for high-LET radiations. This difference can be interpreted by assuming that in hypoxic conditions, because part of the chemical changes are rendered ineffective, lethality is only induced by events which involve larger clusters of energy deposition than required in oxygenated conditions (Barendsen 1967). This stronger clustering is much less efficiently produced by low-LET radiation than by high-LET radiations.

To evaluate the influence of hypoxia more quantitatively, it is of interest to compare the corresponding effective cross-sections, which are also presented in Figure 1, as a function of LET (Barendsen 1967). It can be concluded that the cross-section for oxygenated cells increases most steeply between 50 and 80 keV/ μ m, while for hypoxic cells the steepest increase is observed between 80 and 120 keV/ μ m. The maximum value of about 35 μ m² is the same for both conditions of exposure. Thus the curve of the cross-section for lethal effects in hypoxic cells is

†The notation with the parameters a_1 and a_2 is preferred in this mathematical formula instead of α and β as used by other authors because the latter symbols are associated with many other phenomena in physics, biology and statistics, e.g. alpha- and beta-particles, alpha-PLD and beta-PLD, etc.

Figure 1. *RBE* (circles) and cross-sections (triangles) as a function of LET for inactivation of T-1 cells of human origin in culture, irradiated in oxygenated conditions (open symbols) or in hypoxic conditions (closed symbols). Data points pertain to a_1 calculated on the assumption that survival curves can be described by $s(D)/s(0) = \exp - (a_1 D + a_2 D^2)$. The effective cross-sections were calculated from a_1 as the inverse of the associated particle fluences, corresponding to the dose required to yield an average of one lethal event per cell. Data from Barendsen *et al.* (1963) and Barendsen (1964, 1967).

shifted towards higher LET as compared with oxygenated cells. A particle of $90 \text{ keV}/\mu\text{m}$ passing through a cell in hypoxic condition is associated with a cross-section about equal to the cross-section obtained with a particle of $60 \text{ keV}/\mu\text{m}$ for oxygenated cells. This shift can be interpreted as a reduction in effective local damage by 30–40%, caused by the absence of oxygen in the cells. Thus a larger *OER* of 2–3, e.g. for low-LET radiations, can be explained by a relatively modest increase in the requirement for local clustering of damage.

The cross-section curves can provide yet another type of basic insight. From a comparison of the maximum cross-section of $35 \mu\text{m}^2$ with the cross-sectional area of the cell nucleus of about $70 \mu\text{m}^2$, and consideration of the packing of DNA in the cell nucleus, the suggestion has been derived that, although the probability for induction of cell reproductive death is only about 0.5 per particle, the number of DNA dsb induced by an alpha particle passing through a cell nucleus may be as large as 20–40 (Barendsen 1990, 1994a). This is in agreement with evidence from other types of studies, suggesting that most DNA dsb in mammalian cells are repaired (Radford 1986).

4. LET dependence of sublethal damage (SLD)

The frequency of sublethal lesions, which can interact to cause mammalian cell reproductive death, is represented by the square root of a_2 in the linear-quadratic formula. Sublethal lesions are produced not only by low-LET radiations but also by radiations of high LET, which yield survival curves that are indistinguishable from exponential. This has been quantified in experiments in which high-LET radiations were sequentially combined with low-LET radiations (Bird *et al.* 1983, McNally *et al.* 1988, Barendsen 1993). The *RBE* of SLD can be derived from the square roots of a_2 's calculated for survival curves for ionizing particles with $\text{LET} \leq 25 \text{ keV}/\mu\text{m}$. For larger LETs experiments on the interaction of damage from high-LET particles with SLD from low-LET X-rays have yielded more accurate *RBE*s. The *RBE*–LET relationship for SLD derived from both types of data has been shown to increase by a factor of at most 2–3 between 10 and $100 \text{ keV}/\mu\text{m}$. As illustrated schematically in Figure 2, this relationship is very similar to the *RBE* for induction of double-strand breaks in DNA (Barendsen 1993). It has been hypothesized that a fraction of the DNA dsb constitute the sublethal lesions, and that these sublethal lesions are equivalent to the locally multiply damaged sites (LMDS), suggested by Ward (1990) to be associated with DNA dsb (Barendsen 1994).

5. LET dependence of potentially lethal damage (PLD)

Potentially lethal damage has been detected experimentally as a type of lesion that is subject to removal or expression, depending on conditions to which cells are exposed after irradiation. In particular, by maintaining cells in a resting phase or by temporary impairment of cellular metabolism after a given dose of radiation, repair of PLD results in enhancement of clonogenic capacity. Iliakis (1988) has reviewed evidence suggesting that at least two types of conditions can affect the expression of PLD: (1) conditions that reduce the effectiveness of a radiation dose by preventing fixation of PLD and thereby allowing repair to proceed, and (2) conditions that increase the effectiveness of a dose by fixation of PLD that might have been repaired in standard post-irradiation conditions. PLD has been shown to contribute to the induction of damage represented by the linear term as well as the quadratic term in the LQ formalism (Fertil *et al.* 1988). The dependence of PLD on LET has been studied experimentally by Yang *et al.* (1985) for plateau-phase C3H 10T1/2 cells irradiated with

Figure 2. Schematic representation of RBE -LET relationships for the different types of damage in mammalian cells which contribute to cell reproductive death: STLD = single track lethal damage caused by individual ionizing particles and their associated secondaries, $STLD_{(unr)}$ = STLD that remains effective after the component of PLD is repaired, PLD = potentially lethal damage, that contributes to the linear as well as to the quadratic term in the dose-response relationship, SLD = sublethal damage that determines the quadratic term in the dose response relationship, DNA dsb = DNA double-strand breaks, DNA ssb = DNA single-strand breaks.

heavy ions of a wide range of LETs, and by Bertsche and Iliakis (1987) for Ehrlich ascites tumour cells irradiated with various light ions. These data have been analysed and interpreted recently with respect to the influence of LET on the two parameters of the LQ formula (Barendsen 1994a). The differences of the corresponding a_1 values for immediate plating and for delayed plating respectively, were calculated to derive the linear parameters a_1 for PLD as a function of LET. From this analysis the deduction was made that the RBE for the component of single track lethal damage (STLD), which remains effective after PLD had been eliminated by repair during a delay in plating, is significantly stronger dependent on LET by a factor of about 2, attaining values in the range of 10–20 as compared with the total STLD derived from data on immediate plating. This is illustrated schematically in Figure 2. By contrast, the values of a_1 for the PLD component of STLD, which is subject to repair, do not increase with LET by more than a factor 2–3. This PLD component shows

a similar dependence on LET as SLD and DNA dsb. In a similar way as for STLD, the SLD was analysed to distinguish a component of PLD represented in the quadratic term. It was concluded that the RBE values of the square root of the parameter a_2 did not differ for immediate and delayed plating respectively, and that for both conditions a similar dependence on LET was obtained as for DNA dsb. This supports the hypothesis that sublethal lesions are a subset of the DNA dsb.

These deductions concerning different contributions of various components of biological damage to the expression of cell reproductive death in mammalian cells, are compatible with the suggestion of Goodhead (1989) that several classes of initial physical damage can be distinguished. These classes range from sparse single ionizations which are relatively ineffective, to small and moderate clusters produced by track ends of electrons or delta-rays from fast nuclei, to large clusters caused by high-LET particles, and finally to very large clusters unique to very densely ionizing particles. The results discussed here indicate that a significant part of radiation damage, even from low-LET electrons, can cause STLD that is not repaired by delayed plating. In addition, high-LET radiations are shown to produce, albeit with a relatively low RBE , the same SLD and PLD as is produced by low-LET radiation.

6. Implications of insights derived from RBE -LET relationships for radiotherapy

The identification of two distinct components of damage causing cell reproductive death which can be induced by single-particle tracks, provides the possibility to interpret differences among cell types with respect to their radiosensitivity and RBE . Stem cells in various types of normal tissues are known to exhibit significant differences in sensitivity to low-LET radiations as well as in RBE of high-LET radiations. For instance, bone marrow stem cells are more radiosensitive than stem cells of skin or intestine in the same animal, notwithstanding the identical DNA content. Associated with the higher sensitivity, RBE of high-LET radiations for the induction of lethal events in bone marrow stem cells are generally low. An extreme example is provided by cells from patients with ataxia telangiectasia (AT). These cells show little PLD repair. RBE -LET relationships for AT cells show a maximum RBE of about 2 at 100 keV/ μ m (Cox 1982). On the basis of the present analysis yielding two distinct components of damage in the linear term, it can be suggested that for cells with a high sensitivity, PLD or PLD-like damage provides a large contribution to the linear term of

cell reproductive death, directly associated with the low *RBE* for this type of damage. From an analysis of published survival curves of cells of human origin, Fertl *et al.* (1988) suggested that an important part of the radiation damage contributing to the initial slope of survival curves is repairable, with repair of this PLD enhancing differences among cell strains. Differences in *RBE* can in this context be ascribed to differences in the contributions of the repairable and unreparable components of STLD which differ in their dependence on LET.

A further interpretation of experimental data can be derived which relates to the *RBE* of high-LET radiation for damage to late-responding tissues. It can be hypothesized that the contribution of the PLD-like component of STLD depends on the rate of proliferation of critical cells in tissues. Late responding tissues contain a large fraction of non-cycling cells and are generally characterized by low a_1/a_2 ratios, possibly associated with a low a_1 , due to a more efficient repair of the PLD component of the linear term. A smaller contribution of PLD would also be expected to yield a higher *RBE*. This hypothesis is consistent with the observation that *RBE* of fast neutrons for damage to late-responding tissues are generally larger than for early responding tissues. In late-responding tissues many cells are non-cycling or have a long life span, associated with ample time available for repair of PLD.

A large contribution of PLD or PLD-like damage could also be responsible for low *RBE* of fast neutrons for responses of some types of tumours. For slow-growing tumours frequently high *RBE* were obtained in clinical studies (Battermann *et al.* 1981). If the hypothesis is correct that in slow growing tumours the high *RBE* of fast neutrons is due to a large component of non-cycling cells which repair PLD efficiently, than an important suggestion can be derived with respect to the application of fast neutrons. In several types of tumours an increased rate of repopulation of surviving cells has been suggested to start after a number of dose fractions has induced sufficient cell death to evoke a proliferative response (Fowler 1989). In these rapidly proliferating cells less repair of PLD might occur and as a consequence of the large component of PLD remaining effective, the *RBE* of high-LET radiations might be lower. Thus the application of high-LET radiation might be more beneficial in the first few weeks of a protracted treatment, when more cells are in a resting state, than in the later weeks. A further suggestion which can be derived from the identification of two components contributing to STLD, is of interest with respect to the application of predictive testing for radiosensitivity to standard treatments with low-LET radiations,

using cells cultured from tumours in patients. The variability of the contribution of PLD suggests that it is not sufficient to assess the radiosensitivity of exponentially growing cells in culture, but that assays are also required to measure the capacity of the tumour cells for repair of PLD, e.g. by analysis of the radiosensitivity of cells in plateau phase, using immediate and delayed plating procedures, or in future developments by methods of molecular biology. This information would be important to predict the responsiveness of slow-growing human tumours to X-rays, as well as the *RBE* of high-LET radiations.

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