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A linear-quadratic model of cell survival considering both sublethal and potentially lethal radiation damage

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Summary

We assessed the dose-dependence of repair of potentially lethal damage in Chinese hamster ovary cells x-irradiated in vitro. The recovery ratio (RR) by which survival (SF) of the irradiated cells was enhanced increased exponentially with a linear and a quadratic component, namely ξ and ψ: $RR = e^{\xi D + \psi D^2}$. Survival of irradiated cells can thus be expressed by a combined linear-quadratic model considering four variables, namely α and β for the capacity of the cells to accumulate sublethal damage, and ξ and ψ for their capacity to repair potentially lethal damage: $SF = e^{(\xi - \alpha)D + (\psi - \beta)D^2}$.

Introduction

Simultaneous repair of sublethal (SLD) and potentially lethal damage (PLD) occurs in confluent, density-inhibited plateau-phase cultures of cells irradiated in vitro [5,6,8,13,26], as well as in vivo [4,10]. It has therefore been suggested that not just repair of SLD but also PLD could play a role in clinical radiation therapy [3,21–24]. However, several investigators and clinicians doubt the significance of PLD repair in radiotherapy for several reasons. One of them is that repair of PLD is usually determined at doses that are about 2–5 times higher than the doses per fraction used in radiation treatment protocols so that it is difficult to judge its impact in the clinical situation. We therefore assessed the dose-dependence of PLD repair in Chinese hamster ovary (CHO) cells x-irradiated in vitro and developed a mathematical model for the survival of x-irradiated cells which considers both SLD and PLD.

Materials and methods

Chinese hamster ovary (CHO-K1) cells were grown in Optimem 1 (Gibco, Cat. No. 041-01985 M) supplemented with 5% Fetal Calf Serum (Gibco, Cat. No. 011-06290 M), and 1% Penicillin-Streptomycin solution (Gibco, Cat. No. 043-05140H). Cells were maintained in 60-mm Falcon Primaria tissue culture dishes under standard incubator conditions (humidified, 37 °C, 5% CO_2). The cells were grown to confluence (2.5 Mio. cells/dish). The medium was then replaced by serum-free fresh medium 24 h before irradiation. Cells were irradiated at room temperature in an Oris IBL 637 Cesium irradiator yielding 662 keV gamma rays at a dose-rate of 83.3 cGy/min. The doses were: 1, 2, 3, 4, 6, 8, 10, 12, and 14 Gy. The cells were trypsinized and subcultured at low density either immediately following irradiation to determine survival (SF_i) or after a delay of 24 h confluent holding time in

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the incubator (SF_d). Survival was measured with the routine colony-forming capacity assay. We calculated the recovery ratio (RR) for each dose, expressing the relative increase in survival as a result of PLD repair. The value was calculated from the mean survival fractions determined from three individually treated dishes each for dose, time of subculture and experimental day. Per experimental day, plating efficiencies and RR at up to nine doses were determined. RR was calculated as follows:

$$RR = SF_d \cdot SF_i^{-1} \quad (1)$$

Statistical analysis was carried out with the Statview software package on a Macintosh SE/30.

Results and discussion

Plating efficiencies of the unirradiated cells varied between 48 and 80%. During the 24 h following radiation, the plating efficiency of the unirradiated cells was reduced by an average of 10%. Figure 1 shows the RRs in confluent cultures of CHO-K1 cells as a function of the dose. When the data were fitted to a linear quadratic model, there was an excellent correlation: $RR = 0.9960 \cdot e^{0.071D + 0.011D^2}$ (R squared = 0.911). This fit predicts a recovery ratio of 0.996 for unirradiated cells, and is thus less than 1% from the real value of 1.00.

Such a linear quadratic dose-dependence of PLD repair has previously been suggested for a number of human tumor cell lines irradiated in vitro [12]. More recently, it has also been observed in exponentially growing Chinese hamster V79 cells [17], and by retrospective analysis in 86 human cell lines and strains [2]. Similar data can be found by retrospective analysis of previously published survival data (e.g., data from [1,4,5,9].

These findings allow development of a mathematical model for the survival of x-irradiated cells that considers PLD repair in addition to SLD repair. We suggest to assign the greek letters ξ ("zeta") to characterize the linear, and the greek letter ψ ("psi") to express the quadratic component of the impact of PLD repair on survival:

$$RR = e^{\xi D + \psi D^2} \quad (2)$$

Kellerer and Rossi [7] proposed that survival of cells exposed to low LET radiation and not allowed to repair PLD (SF_i) can be expressed with a linear-quadratic model:

$$SF_i = e^{-\alpha D - \beta D^2} \quad (3)$$

Since $RR = SF_d \cdot SF_i^{-1}$ (1), survival after repair of PLD (SF_d) is:

$$SF_d = RR \cdot SF_i = e^{\xi D + \psi D^2} \cdot e^{-\alpha D - \beta D^2} \text{ (2), (3)}$$
$$= e^{(\xi - \alpha)D + (\psi - \beta)D^2} \quad (4)$$

Overall survival (SF) of a cell population that has been allowed for repair of both SLD and PLD can thus be described as:

$$SF = e^{(\xi - \alpha)D + (\psi - \beta)D^2} \quad (5)$$

These findings have importance for the interpretation of correlations between radiosensitivity and repair of both SLD and PLD determined in vitro, and the clinical outcome. They strengthen the notion that not just α and β – expressing the capacity of the cells to accumulate sublethal damage – are important, but also ξ and ψ – expressing the capacity to repair potentially lethal damage.

The radiosensitivity of tissues is determined by their capacity to accumulate and repair radiation damage. Two components, α and β express the capacity of irradiated cells to accumulate SLD in the linear-quadratic model of cell survival developed by Kellerer and Rossi [7]. This model has been very helpful to understand the biological effects of radiation. However, it does not consider the impact of PLD repair. Contribution of PLD repair to survival following a single fraction of a dose applied in radiotherapy may usually vary between a factor of one and two only and is thus difficult to demonstrate. However, it is important to bear in mind that small fractional events may have a significant impact when the number of fractions is high.

Some arbitrarily chosen values for RR at 2 Gy (RR_{2Gy}) are listed in the left column of Table I. Based on evidence that the capacity for PLD repair is not reserved to the non-cycling cells in tumors

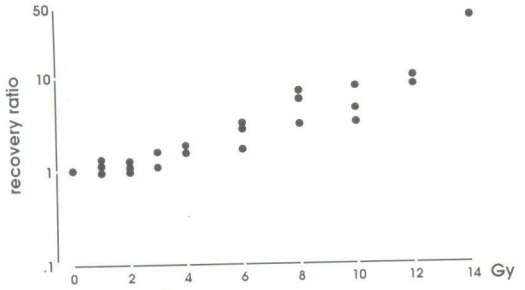

Fig. 1. RRs in confluent cultures of CHO cells as a function of dose.

TABLE I

Arbitrarily chosen values for PLD repair following a dose of 2 Gy (left column) and an estimate of the resulting recovery after 30 such fractions (right column) based on the assumption that recovery after each dose remains the same.

RR_{2Gy}	RR_{60Gy}
1.01	1.35
1.05	4.32
1.10	1.74×10^1
1.15	6.62×10^1
1.20	2.37×10^2
1.25	8.08×10^2
1.33	5.20×10^3
1.50	1.92×10^5
1.75	1.95×10^7
2.00	1.07×10^9

[11,14–17,19,20,25], we calculated values for the total RR (RR_{60Gy}) resulting from PLD repair occurring after each of 30 fractions of 2 Gy resulting in a total dose of 60 Gy (Table I, right column). Since only 1–10 non-inactivated cells are responsible for recurrence in tumors that have at least a 1% chance for permanent local control [18], it is evident that minor tumor-specific inhibition of PLD repair could lead to drastic improvements in terms of permanent local control.

Interestingly, the resulting formula of our model (5) is of linear-quadratic nature. It comes therefore as no surprise, that the linear-quadratic model [7] has been so successful in evaluating and predicting the biological effects of fractionated radiation. However, conceptually it is of importance to note that any apparent α/β ratio in irradiated tissue reflects not just the capacity of the irradiated cells to repair SLD, but also PLD.

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