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Authors
YOHEM, KARIN H
SLYMEN, DONALD J
BREGMAN, MARVIN D
et al.

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Radiosensitivity of Murine and Human Melanoma Cells: A Comparative Study With Different Models

KARIN H. YOHEM,[1] DONALD J. SLYMEN,[2] MARVIN D. BREGMAN, AND FRANK L. MEYSKENS, JR.[3]

Department of Internal Medicine and Cancer Center, University of Arizona, Tucson, Arizona 85724

The in vitro radiosensitivity of one murine melanoma cell line (Cloudman S91 CCL 53.1) and three human melanoma cell strains (C8146C, C8161, and R83-4) were studied. Cells were irradiated by single dose X-rays and plated either in agar or on plastic. The survival curves were fitted by the single-hit multitarget, two-hit multitarget, and quadratic models. Multiple comparisons of the residual sum of squares suggested that the two-hit model was clearly inferior to the single-hit and quadratic models. No statistically significant difference was suggested for either the single-hit or quadratic models. Furthermore, on examination of the differences in correlations between the observed and predicted values, the residual plots (observed minus predicted over dose) failed to suggest a clear advantage of either the single-hit multitarget or the quadratic models. Either model could be recommended for analysis of in vitro radiation data.

Key words: Ionizing radiation, X-irradiation, Clonogenic assay

INTRODUCTION

The human tumor clonogenic assay described by Hamburger and Salmon (1977) has been utilized by investigators studying the efficacy of therapeutic agents for several tumor types. The study of radiation survival of cells grown on plastic began in 1956 with the classic experiments of Puck and Marcus. Together both of these assays have been used in studying the response of tumor cells to a wide variety of agents. Particularly pertinent to our research are those studies that use these assays for monitoring the effects of radiation, chemotherapeutic agents, and biological response modifiers on human malignant melanoma.

Interlaboratory differences between cloning systems, radiation protocol, and statistical analysis used to analyze survival curves have hindered the acquisition of reproducible data to delineate radiation response of human melanoma (Barranco et al., 1971; Courdi et al., 1981; Rofstad and Brustad, 1981, 1983; Selby and Courtenay, 1982; Smith et al., 1978; Weichselbaum et al., 1980; Weininger et al., 1978). In addition, there may be differences in inherent radiosensitivity for different cell lines of one tumor type (Fertil and Malaise, 1981). The objective of the current study was to analyze three models—single-hit multitarget, two-hit multitarget, and quadratic—as to best fit of our radiation data (Yohem et al., 1988). Our results were similar to those of Fertil et al. (1980), which showed that the quadratic model fit the radiation data well. Furthermore, we found that the single-hit multitarget model also fit the radiation data. However, our findings were dissimilar to those for Chinese hamster cells studied by Millar et al. (1978), in which all three models fit the data equally well. We conclude that either the single-hit multitarget or the quadratic models can be used for analysis of in vitro melanoma radiation data.

MATERIALS AND METHODS

Maintenance of CCL Murine Melanoma Cell Line

The Cloudman S91 murine melanoma clone CCL 53.1 was obtained from the American Type Culture Collection, Rockville, MD, and has been maintained by serial transplantation in DBA/2J mice. The tumors were harvested, and single-cell suspensions were obtained as previously described (Bregman et al., 1982). CCL 53.1 cells readily formed a monolayer and were subsequently subcultured. All experiments were performed on cells that had been

Address reprint requests to Karin H. Yohem, Ph.D., Department of Anatomy, College of Medicine, University of Arizona, Tucson, AZ 85724.

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[1]Present address: Department of Anatomy, College of Medicine, University of Arizona, Tucson, Arizona 85724.

[2]Present address: Graduate School of Public Health, San Diego State University, San Diego, California 92182.

[3]Present address: Department of Hematology/Oncology, University of California, Irvine, Cancer Center, 101 The City Drive, Building 44, Route 81, Orange, California 92668.

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subcultured no more than 10 times after isolation from mouse melanomas.

Preparation and Culture of Cells from Patient Biopsies

The general approach to the preparation of cell suspensions has been extensively described elsewhere (Meyskens et al., 1981).

Establishment and Culture of Human Melanoma Cell Strains

Tumor cells were cultured as monolayers in flasks. At confluency, cells were either subcultured or cryopreserved. Cells were checked periodically for mycoplasm. Cell strains that were utilized in these experiments were subcultured less than 10 times. R83-4 was a subclone of patient biopsy derived from cells that formed colonies in plates that had been irradiated with a dose of 10 Gy X-rays. Cells were pooled from the plucked colonies and the cell strain was established as described above.

Soft-agar Bilayer Assay

The soft-agar assay has been described extensively elsewhere (Meyskens et al., 1981). The number of cells to be plated was within the linear range in the relationship of "cells plated" versus "colonies formed" that was determined for each cell strain prior to radiation studies. Cells plated were in the exponential phase of the growth curve. In our studies, plating efficiencies were 30.1–38.6% for the murine melanoma CCL 53.1 and 10.6–17.4%, 10.2–19.1%, and 2.1–2.9% for the human cell strains C8146C, C8161, and R83-4, respectively. Cells were incubated in a well-humidified 5% CO_2 and 95% air atmosphere at 37°C for two weeks. The agar and monolayer experiments were performed at the same time from cells that were divided into two portions, one plated in agar and the other in monolayer. Each experiment was done in triplicate.

Monolayer Assay

The monolayer assay has been described extensively elsewhere (Puck and Marcus, 1956; Elkind and Whitmore, 1967; Steel and Courtenay, 1983). Number of cells to be plated was within the linear range in the relationship of "cells plated" versus "colonies formed," was determineed for each cell strain prior to these radiation studies, and yielded 100–335 colonies per control plate. Cells plated were in the exponential phase of the growth curve. In these studies, plating efficiencies were 51.7–71.0% for the murine melanoma CCL 53.1 and 25.2–27.3%, 9.7–20.0%, and 3.0–3.6% for the human cell strains C8146C, C8161, and R83-4, respectively.

Radiation

Cells were irradiated by a single dose of X-rays generated by a Varian Associates 18 MeV linear accelerator operating at 10 MeV and yielding a dose of 5.0 gray (Gy) per minute. A 2.0 cm thick bolus was placed between the source of irradiation and the target. A source to target distance of 100 cm was used, and the cells were irradiated at ambient temperature under normal atmospheric conditions. All radiation dosages and dosimetry readings were provided by the Department of Radiation Oncology of the University Medical Center, Tucson, AZ.

Model Selection, Estimation, and Comparison of Survival Curves

Survival data were calculated according to standard radiobiological methods (Puck and Marcus, 1956; Elkind and Whitmore, 1967; Steel and Courtenay, 1983). There were twelve replicates per control and six replicates per experimental dose per experiment. D_0 values were estimated from the curve fitted by the one-hit multitarget model. For each replicated experiment the observed survival data consisted of the mean proportion surviving per dose expressed as a proportion of the control mean.

Although several mathematical models have been proposed for analyzing survival data, we chose three 2-parameter models for further analysis. The intent was to fit each of the models to each of the 8 sets of survival data and choose the model which consistently provided the best fit. The following models described in Fertil et al. (1980) were examined:

1. one-hit multitarget

$$S(D) = 1 - \{1 - \exp(-D/D_0)\}^n$$

2. two-hit multitarget

$$S(D) = 1 - \{1 - \exp[-D/D_0(1 - D/D_0)]\}^n$$

3. quadratic

$$S(D) = \exp(-\alpha D - \beta D^2)$$

where D is the experimental dose, and D_0, n, α, and β are parameters to be estimated from the data. Estimation was carried out with nonlinear least squares regression (Draper and Smith, 1981) using the SAS statistical package (SAS Institute Inc., 1985).

For each fitted curve the residual sum of squares (RSS) was calculated, which provided a measure of the discrepancy between observed (S) and predicted ($\hat{S}$) values as shown in the equation below:

$$RSS = \sum_{i=1}^{k} (S_i - \hat{S}_i)^2$$

where k denotes the number of observations. Since each model has 2 parameters, one criterion for selection was to choose that model with the smallest RSS. Within each cell line and assay method the RSS were ranked from the smallest to largest. Friedman's test and Bonferroni multiple comparisons (Conover, 1980) were used to compare the models with respect to RSS.

TABLE 1. Residual Sum of Squares for Radiation Data of Murine and Human Melanoma Cells Fitted by the Single-Hit Multitarget, the Two-Hit Multitarget and the Quadratic Models*

Cell	Assay	Model		
		Single-hit	Two-hit	Quadratic
CCL	Monolayer	0.259 (1)[a]	0.345 (3)	0.274 (2)
	Agar	0.836 (1)	0.914 (3)	0.870 (2)
C8146C	Monolayer	0.302 (1)	0.445 (3)	0.331 (2)
	Agar	0.880 (2)	1.023 (3)	0.849 (1)
C8161	Monolayer	0.221 (2)	0.224 (3)	0.203 (1)
	Agar	0.347 (1)	0.486 (3)	0.368 (2)
R83-4	Monolayer	0.193 (1)	0.213 (3)	0.196 (2)
	Agar	1.238 (1)	1.615 (3)	1.385 (2)

*Friedman's test was used to compare the ranks of the residual sum of squares among the 3 models which was highly significant ($P < 0.001$).
[a]The number in parentheses represents the rank of the residual sum of squares from the smallest (1) to the largest (3) within cell type and assay method.

RESULTS

The residual sum of squares for each model for each of the 8 sets of survival data are displayed in Table 1. Clearly, the two-hit multitarget model consistently had the largest RSS, suggesting that this model yielded the poorest fit to the radiation data. Bonferroni pairwise comparisons confirmed that the two-hit model was inferior to the quadratic and single-hit models. Differences between the quadratic and single-hit models were not statistically significant, suggesting a comparable fit to the data. Figure 1 contains a graphic representation of the fit of each of these three models to a representative set of experiments. Similar results were obtained for all other data.

The two-hit multitarget model consistently yielded higher RSS values and, therefore, was removed from further analysis. Correlation coefficients were calculated between observed and predicted values for the two remaining models (see Table 2). All correlations were at least 0.9 indicating that both models fit the data well. Graphical methods were used to further evaluate the fit of each model. The residual values ($S-\hat{S}$) were plotted over dose to observe any systematic patterns suggesting lack of fit. However, both models provided an adequate fit with neither model showing marked superiority over the other.

DISCUSSION

We irradiated murine and human melanoma cells and analyzed the data using three different models. We determined the residual sum of squares and the correlation of the observed with predicted values of the data using the two-hit multitarget model, the single-hit multitarget, and the quadratic models. Both the single-hit multitarget and quadratic models fit the data equally well while the two-hit multitarget model was less accurate. There may be biological considerations, especially in effects of high dose versus low dose irradiation, which would make one model preferential over the other.

According to Fertil et al. (1980) the quadratic model is well adapted for describing the initial part of the survival curve. In experiments using the lowest dosages of radiation (1–2 Gy) and/or limited to 2 or 3 decades of cell kill, they propose that the quadratic model be used because the single-hit target model minimizes the effects of radiation at the initial part of the curve, especially in the dose range of 1–2 Gy.

Since our radiation data spans three decades of kill and we were interested in the effects of low dose radiation, we used the quadratic model for analyzing our data (Yohem et al., 1988). Fertil and coworkers (1980) found that the quadratic model fit their data most accurately. They analyzed the survival curves for six cell lines (including the MEWO melanoma cell line). A controversy remains, however, concerning the biological significance of the parameters α and β, with regards to the importance of the repair of damage versus the importance of type of damage. The α-component is the linear, exponential component; β is the bending component. It has been theorized that the value α and the ratio of α/β corresponds to the dose at which accumulation of sublethal damage results in the increase of lethality to more than half the population, i.e., the sublethal damage is not repaired (Chadwick and Leenhouts, 1973). More recently, Steel and Peacock (1989) have proposed that the incidence of the α-type damage in cells may be the principal reason why the most responsive human tumors are so sensitive. Furthermore, they propose that the inherent radiosensitivity of the tumor is defined by the type of damage sustained by the cells, not the repair of the damage. The α values and α/β values varied considerably for the melanoma cells that we studied (Yohem et al., 1988) which suggested the possibility that differences in radiation sensitivity may be due to differences in the intrinsic radiosensitivity of the cells as well as the assay and mathematical model used to describe radiosensitivity.

TABLE 2. Correlations: Observed with Predicted Values for Radiation Data of Murine and Human Melanoma Cells Fitted by the Single-Hit Multitarget and the Quadratic Models

Cell	Assay	Model	
		Single-hit	Quadratic
CCL	Monolayer	0.95	0.94
	Agar	0.95	0.95
C8146C	Monolayer	0.97	0.97
	Agar	0.90	0.90
C8161	Monolayer	0.96	0.97
	Agar	0.96	0.96
R83-4	Monolayer	0.96	0.96
	Agar	0.95	0.94

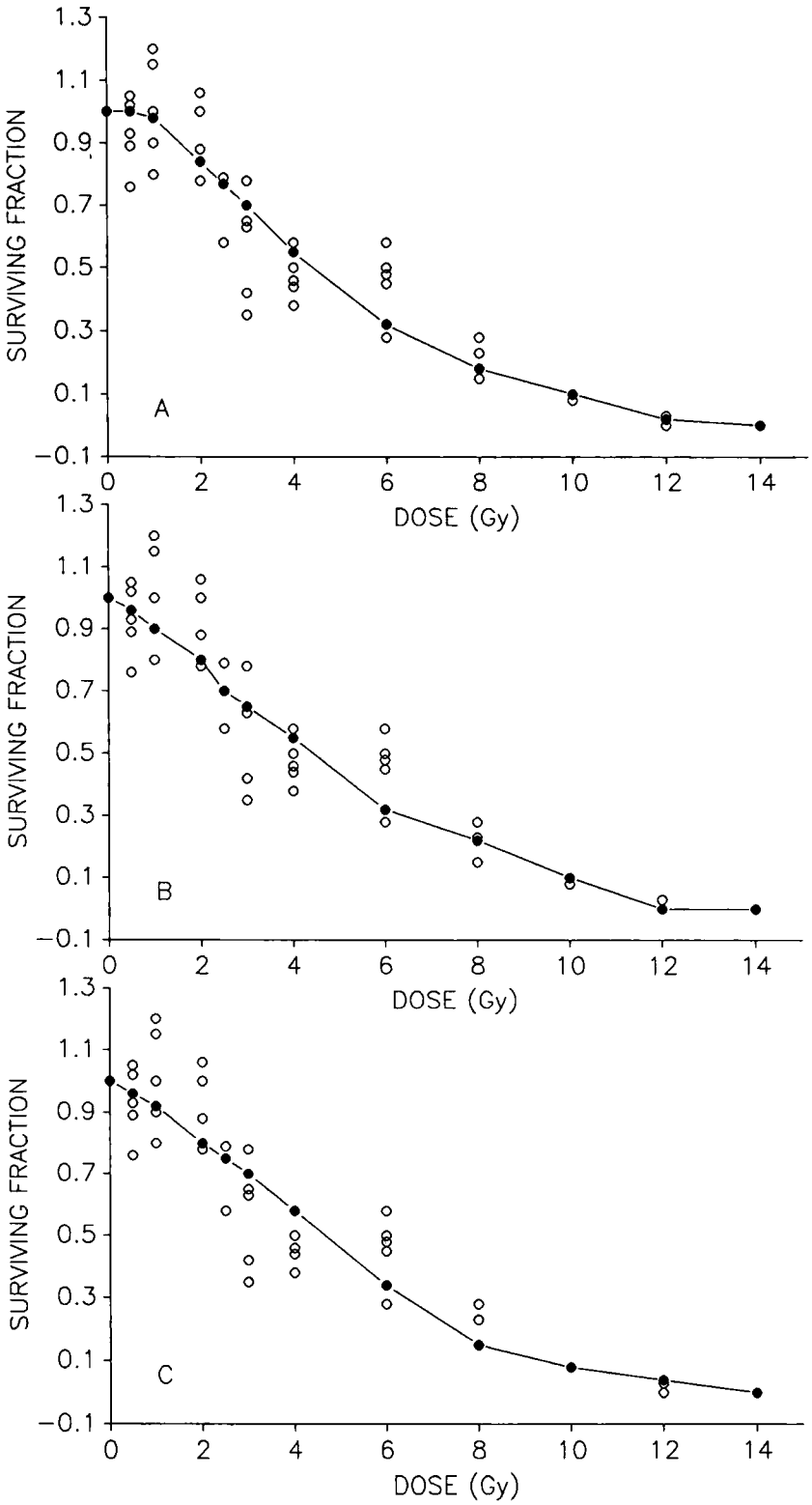

Fig. 1. Radiation survival curves of murine melanoma Cloudman S91 CCL 53.1 cells grown in agar. The observed values are represented by open circles. The predicted values are represented by closed circles. The solid line indicates the predicted survival curve fitted by a mathematical model. A–C are from the same, representative set of experiments. Seventy-seven observed values are hidden. A, Data fitted by the single-hit multitarget model. B, Data fitted by the two-hit multitarget model. C, Data fitted by the quadratic model.

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