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ESTIMATION OF ERRORS ASSOCIATED WITH USE OF LINEAR-QUADRATIC FORMALISM FOR EVALUATION OF BIOLOGIC EQUIVALENCE BETWEEN SINGLE AND HYPOFRACTIONATED RADIATION DOSES: AN *IN VITRO* STUDY

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Purpose: To investigate the reliability of the linear-quadratic (LQ) formalism and the magnitude of errors associated with its use in assessing biologic equivalence between single, high radiation doses and hypofractionated radiation doses.

Methods and Materials: V79 and EMT6 single cells received single doses of 2–12 Gy or two or three fractions of 4 or 5 Gy, each at 4-h intervals. Single and fractionated doses to actually reduce the cell survival to the same level were determined by a colony assay. The α/β ratio was obtained from the cell survival curves. Using the α/β ratio and the LQ formalism, equivalent single doses for the hypofractionated doses were calculated. They were then compared with the actually determined equivalent single doses for the hypofractionated doses. The V79 spheroids received single doses of 5–26 Gy or two to five fractions of 5–12 Gy at 2 or 4-h interval, and then were assayed for cell survival. Next, equivalent single doses for the hypofractionated doses were determined, as were done for the single cells.

Results: The α/β ratio was 5.1 Gy for the V79 single cells and 0.36 Gy for EMT6. In V79, the equivalent single doses for the hypofractionated doses calculated using the LQ formalism were 12–19% lower than the actually measured biologically equivalent single doses. In the EMT6 cells, this trend was also seen, but the differences were not significant. In the V79 spheroids, the calculated doses were 18–30% lower than the measured doses.

Conclusion: Conversion of hypofractionated radiation doses to single doses using the LQ formalism could underestimate the effect of hypofractionated radiation by ≤30%. © 2009 Elsevier Inc.

Linear-quadratic model, equivalent dose, radiosurgery, hypofractionation.

INTRODUCTION

The use of stereotactic irradiation is spreading worldwide, and various new machines and techniques have been developed. Each machine or technique possesses respective characteristics, and, accordingly, various fractionation schedules have been used. Gammaknife stereotactic radiosurgery (SRS) usually provides single-fraction stereotactic irradiation, and frameless linear accelerator-based stereotactic irradiation and the cyberknife can offer, not only single-fraction SRS, but also fractionated stereotactic radiotherapy (SRT). Hypofractionated SRT has been increasingly used as an alternative to surgery for Stage I non–small-cell lung cancer (1–3). For malignant tumors containing hypoxic cells, SRT appears to be more efficient than single-fraction SRS because it takes advantage of the reoxygenation of hypoxic tumor cells during fractionation (4–7). In addition, for sparing of normal tissues, especially late-responding tissues such as the brain and spinal cord, fractionated radiation is expected to be more efficient than with single-fraction radiation (8, 9). Therefore, fractionated SRT appears to be attracting more attention.

In comparing the effects of various fractionation schedules, a formula based on the linear-quadratic (LQ) model $\{n_2d_2/n_1d_1 = [1+d_1/(\alpha/\beta)]/[1+d_2/(\alpha/\beta)]$, where d_1 and d_2 are the fractional doses and n_1 and n_2 are the fraction numbers$\}$ is frequently used. However, this formula should be applied to radiotherapy with many fractions (10, 11). It remains unclear whether the LQ formalism is applicable to radiotherapy with a few fractions. Nevertheless, many clinicians have used the LQ formalism with single or hypofractionated radiation

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schedules (1, 12–14), because no other model is adequate to compare the biologic effects of single and hypofractionated radiation doses. In the present study, therefore, we investigated the reliability of the LQ formalism and magnitude of errors associated with its use when comparing single and hypofractionated radiation doses using *in vitro* single cell lines and spheroids. We evaluated how the biologic effects of hypofractionated irradiation might be misestimated compared with those of single-fraction irradiation using the LQ formalism.

METHODS AND MATERIALS

Cells and spheroid culture

V79 Chinese hamster lung fibroblast and EMT6 mouse mammary sarcoma cells were used. The characteristics of these cell lines have been previously described (15–17). Both cell lines were cultured in Eagle's minimum essential medium supplemented with 12.5% fetal bovine serum. This medium was used throughout the experiments. The cells were subcultured on the day before the single-cell experiments to maintain exponential growth. The methods for culture of the spheroids have been previously described in detail (15, 18). Both V79 and EMT6 cells could be grown as spheroids; however, in a preliminary experiment, no hypoxic fraction could be detected in the EMT6 spheroids. Thus, the EMT6 cell line was not used for the spheroid experiments. To develop V79 spheroids, V79 single cells were plated onto 0.75% soft agar in complete medium as described by Yuhas *et al.* (19). The small spheroids that had formed 7 days after plating on soft agar were transferred into a spinner flask (Bellco Glass, Vineland, NJ) containing 100 mL of Eagle's minimum essential medium. The flask was maintained on a magnetic stirrer (Bellco Glass) operated at approximately 110 rpm in a carbon dioxide incubator. The medium was changed every 3 days thereafter. The V79 spheroids had grown to approximately 0.8 mm in diameter 14–18 days later and were collected using a microdispensor and used for the experiments. Six spheroids were used for each determination.

Irradiation

Irradiation was performed using a 210-kVp X-ray machine (10 mA with 2-mm Al filter; Chubu Medical, Matsusaka, Japan) at a dose rate of 1.8 Gy/min. The dose was calibrated using a RAMTEC 1000 dosimeter (Toyo Medic, Tokyo, Japan). The spheroids were irradiated in culture dishes containing the soft agar as described in the previous section.

Fractionation schedule and assay

In the experiments for single cells, the standard colony assay was used to determine cell survival. After trypsinization, the cell number in the single-cell suspensions was counted using a Coulter counter, and the appropriate numbers of cells were plated. After ≥30 min, irradiation was started. Both V79 and EMT6 single cells received single doses of 0, 2, 4, 6, 8, 10, or 12 Gy, or two or three fractions of 4 or 5 Gy each at 4-h intervals. This interval was chosen from the results of the previous study showing that sublethal damage repair (SLDR) was complete within 4 h (16, 20).

In the spheroid experiments, the potentially lethal damage repair and SLDR were first investigated to determine the optimal intervals between the fractions. V79 spheroids received a single dose of 20 Gy, and the spheroids were assayed for cell survival at 0-, 0.5-, 1-, 2-, 4-, and 6-h intervals. Next, the V79 spheroids received two fractions of 10 Gy at 0-, 0.5-, 1-, 2-, 4-, and 6-h intervals to evaluate SLDR, and the cell survival was evaluated in a similar way. From these experiments, the interfraction intervals and timing for dissociation of spheroids were determined. In additional experiments, the V79 spheroids received a single dose of 0, 5, 10, 15, 20, or 25 Gy, two fractions of 8, 10, or 12 Gy, or three fractions of 6, 7, or 8 Gy at 4-h intervals. They also received single doses of 0, 14, 17, 20, 23 or 26 Gy, four fractions of 5, 6, or 7 Gy, or five fractions of 4, 5, or 6 Gy at 2-h intervals.

After irradiation, the V79 spheroids were dissociated into single cells by treatment with 0.25% trypsin and 0.02% ethylenediaminetetraacetic acid at 37°C for 15 min. Next, the cell number was counted using a Coulter counter, and the appropriate numbers of cells were plated onto culture dishes. In all single-cell and spheroid experiments, the colonies were fixed with 75% ethanol and stained

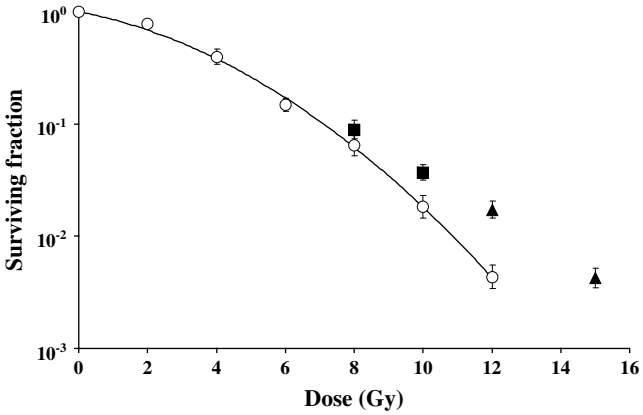

Fig. 1. Dose-survival data for V79 single cells. White circles indicate single fraction; black squares, two fractions (4 Gy × two fractions, 5 Gy × two fractions); black triangles, three fractions (4 Gy × three fractions, 5 Gy × three fractions). Bars represent standard error of four experiments. Approximate equation for survival curve: log (S) = $-0.012D^2 - 0.059D$. Thus, α/β ratio is 5.1 Gy.

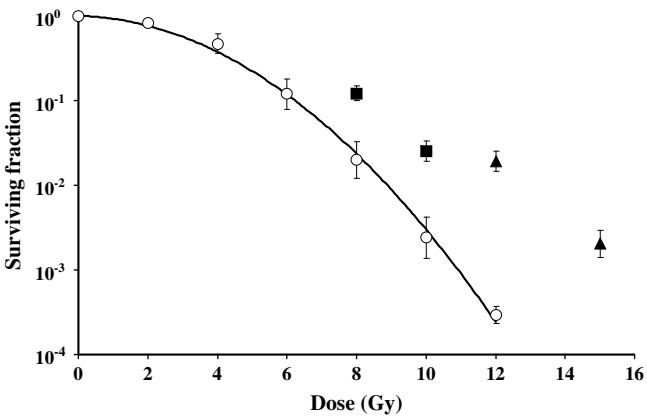

Fig. 2. Dose–survival data for EMT6 single cells. White circles, single fraction; black squares, two fractions (4 Gy × two fractions, 5 Gy × two fractions); black squares, three fractions (4 Gy × three fractions, 5 Gy × three fractions). Bars represent standard error of three experiments. Approximate equation for survival curve is log (S) = $-0.024D^2 - 0.0087D$. Thus, α/β is 0.36 Gy.