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Original Paper

Uniformity of Biological Effect in High-dose Rate Stepping Source Brachytherapy: an *In Vitro* Study

M. Furuta,[1] I. Tsukiyama[1] and Y. Kano[2]

Divisions of [1]Radiation Therapy; and [2]Medical Oncology, Tochigi Cancer Centre, 4-9-13 Yonan, Utsunomiya, 320, Japan

In brachytherapy with a high dose rate of iridium-192, to make a flat isodose surface that covers a target volume, the dwell times of the single stepping source are computer adjusted. However, the dose rates of the irradiation vary with the positions in the volume. To examine this effect, we developed an *in vitro* model of stepping source brachytherapy with a 20 cm long target volume, and determined the uniformity of the biological effect in the volume on two human cancer cell lines (WiDr and A549) plated on a multi-well plate. When the source–cell distance was 10 mm, D1 (radiation dose for 1% survival) was similar in seven positions among the target volume for both cell lines. In contrast, at the 3 mm source–cell distance for one of the two cell lines (A549), D1 in the centre of the volume was significantly higher than at the periphery, suggesting a milder antitumour effect in the centre. Considering the possible decreased effect in the centre of the target volume, where most cancers clinically exhibit their maximum tumour volume, a minimum increase in radiation dose in the centre may be clinically reasonable, especially when dose reference points are set close to the source.

Key words: brachytherapy, high-dose rate, iridium-192, oesophageal cancer
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INTRODUCTION

BRACHYTHERAPY WITH a single source of high dose rate (HDR) iridium-192 (Ir-192) is now a widely used modality of radiation therapy [1]. The radioactive source is controlled by computer and steps through the catheters of an implant or intracavitary applicators. This makes it possible to optimise the source placement in the catheters in each case. Moreover, radiation exposure to staff is eliminated in remote after loading systems.

To form a flat isodose surface that covers a target volume (a planned volume to which a prescribed dose is administered), the dwell times of the source are generally set to be longer at the periphery of the treatment volume and shorter in the centre, instead of implanting radioactive sources outside the tumour. Therefore, peripheral parts of the tumour are irradiated at higher dose rates for shorter times per given dose, whilst the central part is irradiated at lower dose rates for longer times [2]. Although physically the same dose is eventually given to all parts of the tumour, some clinical radiotherapists have uncertainty about the uniformity of the effects in a long-shaped target volume in a single session of brachytherapy for diseases such as oesophageal cancer [3]. This is because the dose rates in the treatment change sequentially and markedly depending on the distances from dwell positions, and the dose rate and exposure time are known to influence biological effects. Regarding the dose rate effect, Fowler theoretically calculated the biological effects of pulsed brachytherapy (intermittent exposure with a constant higher dose rate) and traditional low dose rate brachytherapy (continuous exposure) and found no significant differences in their effects, as long as the tissue had a long half time of repair from radiation damage [4, 5]. Fowler's theoretical calculations, and those of Brenner and Hall [6], demonstrated that the difference in dose rate and exposure time (dose rate effect) was negligible. However, no experimental or clinical data have been shown for HDR brachytherapy, in which the dose rate changes markedly, as mentioned above. In this study, we developed an *in vitro* experimental model of

Correspondence to M. Furuta.
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optimised HDR brachytherapy, and determined the evenness of the biological effects in a 20 cm long target volume on two lines of human cancer cells plated on a row of a multi-well plate and irradiated in the 'optimised' manner. The aim of this study was to obtain the answer to the clinical questions mentioned above. The study of new radiobiological mechanisms was beyond the scope of this study. For this reason, the experiments were performed under conditions that simulated clinical therapy as closely as possible, although this resulted in some difficulties in quantitative analysis as will be discussed later.

MATERIALS AND METHODS

Cells

Two human cancer cell lines were used in the study: (1) a colon adenocarcinoma line (WiDr); and (2) a lung adenocarcinoma line (A549). The plating efficiencies of the cells were 41% (95% confidence interval (CI) 33–49%) for WiDr cells and 32% (26–39%) for A549 cells.

Radiation source

MicroSelectron-HDR with a single source of Ir-192 (Nucletron Internationals, B.V., The Netherlands) was used. The source strength decays with a half-life of 72 days, and the mean radioactivity of the source used was 5.7 Ci (4.8–6.7 Ci).

Treatment planning

A single straight cylindrical target volume (20 cm in length) was made virtually and a catheter (through which the source stepped) was set in the centre of the target volume. Dose reference points were set 5 mm equidistant along the catheter at 3 or 10 mm distances from the catheter (Figure 1). The radiation doses were calculated at these points. The step length of the source was 5 mm. For the treatment planning, PLATO-BPS version 13.1 (Nucletron Internationals) was used to make a flat isodose surface to cover the target volume. Dwell times of the source in each dwell position were optimised by the dose-point optimisation method (mode: on dose-points only) [2].

Procedures

Logarithmically growing cells were plated into seven wells in a row on a 96-well plate (Corning, Massachusetts, U.S.A.) at 22 h before irradiation. Each well contained 100–1600 cells in RPMI medium (200 µl) according to radiation doses. Considering the symmetry in the treatment volume, the positions of the seven wells (A–G) were set at 0.5, 1.4, 2.2, 4.9, 5.8, 8.5 and 9.4 cm from one end of the active length. In the treatment, the plate was set 3 or 10 mm above the catheter according to the reference point setting, and the Ir-192 source stepped through the catheter and irradiated cells in the wells at room temperature. The plates were sealed with tape during the irradiation. Irradiation took approximately 0.5 min to administer 1 Gy to the entire treatment volume when the source–cell distance was 3 mm, and 2 min when the distance was 10 mm. One plate was prepared as a control in each experiment. The plates were then incubated in 95% air/5% CO_2 at 37°C for 7 (WiDr) and 5 days (A549) following irradiation. The cells were then fixed and stained with crystal violet in methanol and colonies consisting of more than 50 cells were counted. Each experiment was performed in duplicate and repeated three times.

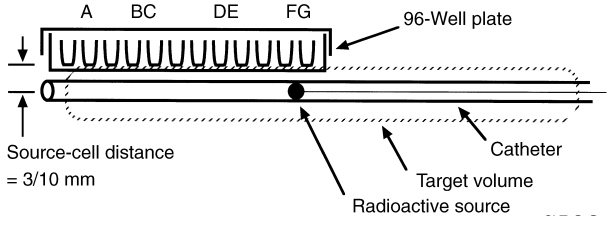

Figure 1. Scheme of experimental setup. A–G are the positions of the wells examined.

Variations in administered dose in a well

The reference points were set on the centre line of the seven wells. Radiation doses vary even in a small well (6.4 mm in diameter) because the dose delivered decreases as the source–target distance increases. In the well, the distance between the catheter and the cells plated at the edge of the well (farthest from the catheter) was 10.5 mm in the 10 mm source–cell distance, and 4.4 mm in the 3 mm setting. Accordingly, the dose delivered to the cells was reduced to approximately 90% of the prescribed dose (the dose at the centre of the well) in the 10 mm setting, whilst the dose decreased to 70% or less in the 3 mm setting.

Variations in dose rate among well positions

Mean dose rates in each well position vary because the dwell times of the source are not the same for each dwell position (optimisation). The difference in dose rate between the wells at the end of the active length (0.5 cm from the end, well A) and in the centre (9.4 cm from the end, well G) is shown in Figure 2. The dose rate is calculated from the dwell time and the well positions when the source activity is 5 Ci.

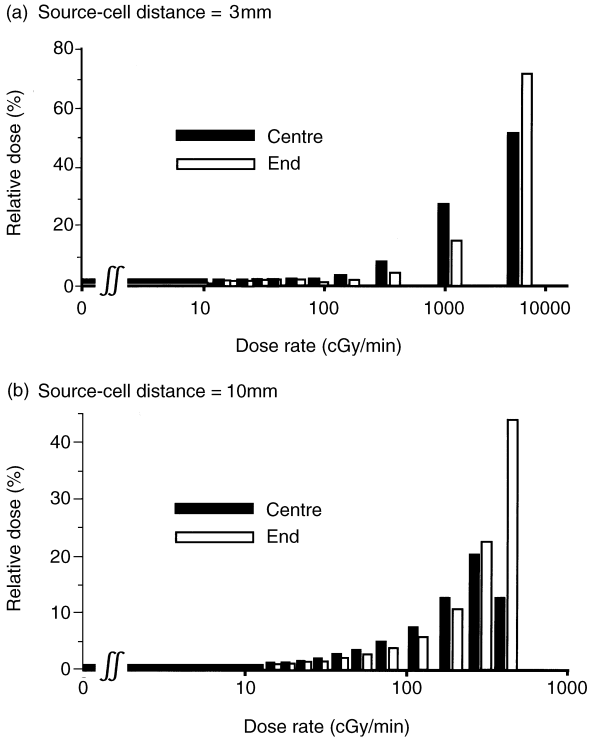

Figure 2. Dose rate distribution at the end (well A) and centre (well G) of the treatment volume in this study. Relative dose (a dose delivered at a given dose rate)/(a prescribed total dose).

When a prescribed dose is delivered to these reference points, the dose rates at which cells are irradiated are higher at the end than at the centre.

Data analysis

Dose–response curves for the seven well positions were drawn and each curve was fitted to a linear-quadratic model ($s = \exp(-\alpha D - \beta D^2)$). The radiation dose resulting in 1% survival (D1) was calculated from the curves, and D1s in the different well positions were compared. Regression analysis to estimate D1 was performed using StatView Version 4.5J (Abacus Concepts, California, U.S.A.). The equation used to obtain a 95% CI is described in the Appendix. The surviving fraction in dose–response curves was statistically compared by Mann–Whitney's *U*-test.

RESULTS

Figure 3 shows survival data of the seven well positions (A–G) for the two human cancer cell lines. The dose–response curves showed little variation among the well positions for WiDr cells and in a 10 mm source–cell distance setting for A549 cells. A moderate change in the dose–response curve among the positions was noted only in the 3 mm distance experiment for A549 cells. The surviving fraction at 10 Gy irradiation was lowest in well B, followed by C and A. The highest surviving fraction was noted in well G. The averages of α and β (of the linear quadratic model) in the seven well positions for WiDr and at the 10 mm setting for A549 are shown in Table 1. At the 3 mm setting for A549, in contrast, the α and β values varied from 0.19/Gy and 0.051/Gy^2 (well B) to 0.31 Gy and 0.013/Gy^2 (well G). When D1 was plotted against well position (Figure 4), no significant differences in D1 were seen among the seven positions for WiDr cells. For A549 cells, D1 values were higher in the centre of the treatment volume than at the end in 3 mm source–cell distance experiments (wells B and C versus G). D1 in well A was also significantly higher than in wells B and C. These differences were not apparent at the 10 mm source–cell distance setting. In Figure 4a, 95% CI at wells D and E were very large, suggesting that the adversely estimated CIs of D1 levels in these wells statistically have little meaning.

Table 1. *α and β values for the two cell lines*

Cell line	3 mm distance α/β (± SE)	10 mm distance α/β (± SE)
WiDr	0.17/0.022 = 7.73 Gy (± 0.92)	0.057/0.037 = 1.54 Gy (± 0.24)
A549	Well B: 0.19/0.051 = 3.69 Well G: 0.31/0.013 = 23.5	0.31/0.021 = 14.8 Gy (± 0.82)

SE, standard error.

DISCUSSION

The purpose of this study was to obtain the answer to the question of intraluminal brachytherapy for diseases such as oesophageal cancer: will all parts of the tumour be irradiated optimally? To answer this question, we created an experimental setting to simulate clinical therapy as closely as possible. The dose delivered to each cell in a well is not consistent as described above. However, tumours encountered in clinics are often large masses. We may consider a circle 6 mm in

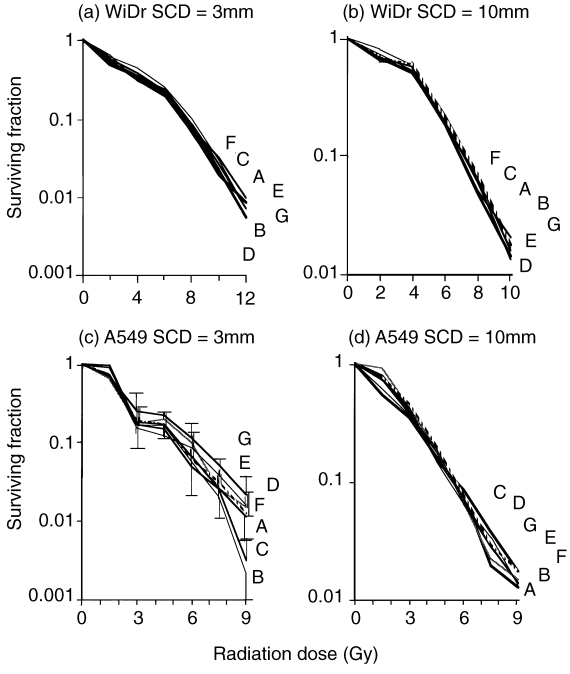

Figure 3. Survival curves for irradiated WiDr (a, b) and A549 (c, d) cells with source–cell distances (SCD) of 3 mm (a, c) and 10 mm (b, d). Error bars (2 standard errors) are shown only in (c) for clarification.

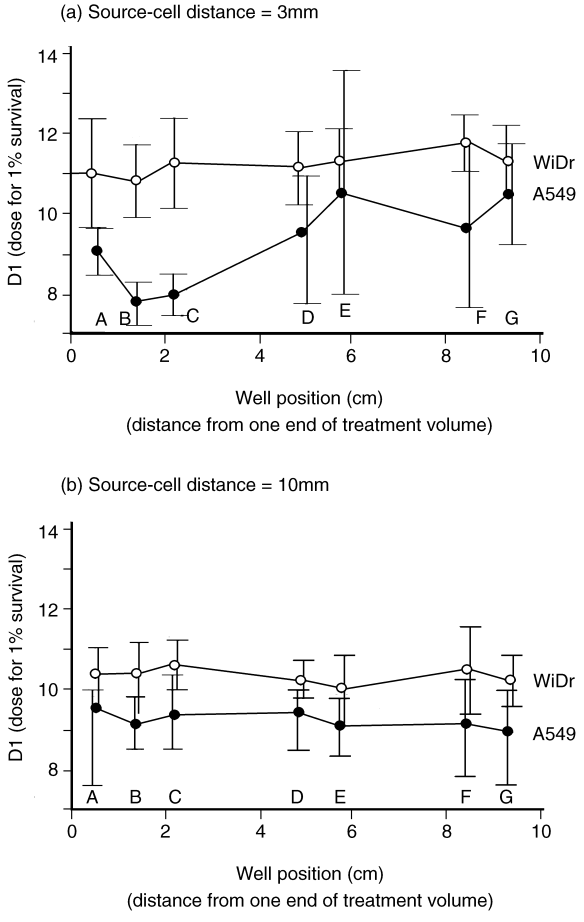

Figure 4. The radiation dose that resulted in a 1% survival (D1) is plotted against the positions of wells (A–G) for WiDr and A549 cells. Source–cell distances (SCD) are 3 mm (a) and 10 mm (b). Error bars are 95% confidence intervals (CI).

diameter as one point and compare several points within a large tumour. In our experiments, the seven wells received the same dose, and their data can be compared with each other.

In one of the two cell lines (WiDr), D1 values were homogeneous among the positions in the target volume. In contrast, in A549 cell at the 3 mm setting, D1 values in the centre of the target volume were revealed to be significantly higher than those at the periphery, suggesting a decreased antitumour effect at the centre. When we compared D1 values in 3 and 10 mm settings in WiDr, D1 values in the 10 mm setting were lower than those in the 3 mm setting in all positions examined, although not to a statistically significant degree (Figure 4). This is explained by the fact that the dose actually delivered to the cells in one well is less in the 3 mm setting than in the 10 mm setting, as mentioned in Materials and Methods. In contrast, in the A549 cell line, D1 levels in the outer wells, A, B and C, at the 10 mm setting were *higher* than those at the 3 mm setting, whilst D1 levels in the inner wells, D, E, F and G, at the 10 mm setting were lower than (or at least the same as) those at the 3 mm setting, as for the data for the WiDr cells. This finding again shows a greater antitumour effect at the periphery of the treatment volume than in the centre at the 3 mm setting for A549.

The dose rates at which the cells were actually irradiated were calculated from the dwell times and well positions. More than 72% of a given dose was delivered at a dose rate of more than 4000 cGy/min at the end of the treatment volume, and 28% of the total dose was administered at dose rates of 1100 cGy/min or less in 3 mm source–cell distance experiments (when the radioactivity of the source was 5 Ci and the heterogeneity of the dose in the well was ignored to simplify calculation) (Figure 2). In contrast, the cells at the centre of the treatment volume received only 52% of the dose at a dose rate of more than 4000 cGy/min. This variance in dose rate might account for the difference in radiation effect seen in A549 at the 3 mm setting.

Why did only A549 cells show heterogeneity for D1? Cell survival, in general, depends on repair capacity for radiation damage. Repair capability depends on the duration of exposure time relative to the half time of the repair, as well as the intrinsic repair capacity of the tissue (ratio α/β in the linear quadratic formula) and dose per fraction [7]. Moreover, when cells are specifically irradiated by pulsed brachytherapy at instantaneous high dose rates close to the dwell positions, the biological effect is influenced by: (1) the dose per pulse; (2) the half time of repair; and (3) the instantaneous dose rate (when the half time is very short) [8]. When the half time of repair is long, the variations in dose rate in question would smooth out before the next pulse of irradiation [4]. In the present study, the dose per fraction and the dose rate were identical between the two cell lines. The half time of repair for A549 cells may be much shorter than that for WiDr cells (10 min or less), at least under conditions similar to those of the present study. The half time of repair in the two cell lines, however, cannot be directly calculated from our data because the calculation needs a constant dose rate irradiation, or at least a constantly decaying dose rate, such as in Au-198 grains [9]. In the literature, Matthews and colleagues showed the repair half time for A549 to be as long as 3 h with α and β values of 0.17 and 0.036 [10]. The repair half time in their study was much longer than we deduced from our results. This may be partly due to the difference in the method of irradiation and partly due to the methodology of calculating the time—they used results of split-dose experiments. We cannot explain why D1 levels in well A were significantly higher than those in wells B and C at the 3 mm setting in A549 cells. Further studies are needed to investigate these issues.

The present study showed that biological effects can differ in a treatment volume, depending on the conditions. However, this finding is noted only in a single cell line, and the conditions of radiation (the active length and source–cell distance) were different from those usually adopted clinically. Clinical extrapolation from our data should be made with caution. However, from a clinical point of view, cancers often exhibit a larger tumour volume in the centre of the lesion than at the periphery. Therefore, to eradicate a tumour and reduce radiation exposure to surrounding normal tissue, a completely flat isodose surface may not always be best for all tumours, and a minimal increase in radiation dose in the centre of the treatment volume might be reasonable in some treatments, especially when dose points are set very close to the source. Another way to avoid possible heterogeneity of the effect is to reduce the dose per fraction and increase the number of treatments [8]. In the treatment of oesophageal cancer, it is impossible to keep a balloon applicator in the oesophagus for a long time, and frequent insertions of the applicator are not a realistic option.

When analysing treatment outcomes, attention should be paid not only to whether the tumour was controlled, but also to the tumour area in the treatment volume from which local recurrence developed or at which severe radiation injury occurred.

1. Nickers P, Kunkler I, Scalliet P. Modern brachytherapy: current state and future prospects. *Eur J Cancer* 1997, **33**, 1747–1751.
2. Van der Laarse R, Prins TPE. Introduction to HDR brachytherapy optimisation. In Mould RF, Battermann JJ, Martinez AA, Speizer BL, eds. *Brachytherapy from Radium to Optimisation*. Veenendaal, Nucletron International B.V., 1994, 331–351.
3. Galelli M, Feroldi P. The time variable dose-rate in HDR stepping source brachytherapy. *Radiother Oncol* 1993, **27**, 173–174.
4. Fowler JF. Time-variable dose rate in HDR stepping source brachytherapy. *Radiother Oncol* 1993, **29**, 355–356.
5. Fowler JF. Why shorter half-times of repair lead to greater damage in pulsed brachytherapy. *Int J Radiat Oncol Biol Phys* 1993, **26**, 353–356.
6. Brenner DJ, Hall EJ. Conditions for the equivalence of continuous to pulsed low dose rate brachytherapy. *Int J Radiat Oncol Biol Phys* 1991, **20**, 181–190.
7. Fowler JF, Mount M. Pulsed brachytherapy: the conditions for no loss of therapeutic ratio compared with traditional low dose rate brachytherapy. *Int J Radiat Oncol Biol Phys* 1993, **23**, 661–669.
8. Fowler JF, Van Limbergen EFM. Biological effect of pulsed dose rate brachytherapy with stepping source if short half-times of repair are present in tissues. *Int J Radiat Oncol Biol Phys* 1997, **37**, 877–883.
9. Thames HD. An 'incomplete-repair' model for survival after fractionated and continuous irradiations. *Int J Radiat Biol* 1985, **47**, 319–339.
10. Matthews JHL, Meeker BE, Chapman JD. Response of human tumor cell lines *in vitro* to fractionated irradiation. *Int J Radiat Oncol Biol Phys* 1989, **16**, 133–138.

APPENDIX
EQUATION FOR CALCULATING A 95% CONFIDENCE INTERVAL FOR D1 LEVEL

When the estimated equation from regression analysis for the dose–response curve is

$$s = \exp\left(-\alpha D - \beta D^2\right)$$

where s is the surviving fraction and D is the radiation dose (Gy),

$$S = \log s = -\alpha D - \beta D^2.$$

When $D = D_0$ and $S = S_0$, the estimated value for S_0 is

$$\hat{S}_0 = -\hat{\alpha} D_0 - \hat{\beta} D_0^2,$$

where $\hat{\alpha}$ and $\hat{\beta}$ are estimated values of α and β.

Then,

$$t = \frac{\mid S_0 + \hat{\alpha} D_0 + \hat{\beta} D_0^2 \mid}{\sqrt{\left(1 + \frac{1}{n} + \frac{\left(D_0 - \overline{D}\right)^2}{S_{DD}}\right) V_e}}$$

where n is the sample number, $\overline{D}$ is the mean value of D, V_e is an unbiased estimate of error variance σ^2, and S_{DD} is the sum of squares of deviations of D or $(Di - D)^2$. The probability is 0.95 that t lies between $\pm t_{n-2,0.05}$, the tabulated 5% point of the t distribution on $n - 2$ degrees of freedom

$$\frac{\mid S_0 + \hat{\alpha} D_0 + \hat{\beta} D_0^2 \mid}{\sqrt{\left(1 + \frac{1}{n} + \frac{\left(D_0 - \overline{D}\right)^2}{S_{DD}}\right) V_e}} \leqq t_{(n-2,0.05)}.$$

When we solve this quadratic inequality, the upper and lower bounds of the 95% confidence interval are obtained.