Simultaneous Intraluminal Thermobrachytherapy: An in vitro Study

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A multi-institutional study on simultaneous intraluminal thermobrachytherapy (SITB) for advanced esophageal cancer was conducted in Japan. In this study, brachytherapy was administered by a small radioactive source stepping through a catheter in the esophagus, and hyperthermia was also applied by an endoesophageal coil. However, experimental or clinical findings on the spatial distribution of its antitumor effects around the esophagus are not available. Therefore, we developed an in vitro model of SITB using a high-dose-rate iridium-192 stepping source and two human cancer cell lines (WiDr and A549), and determined the spatial distribution of the antitumor effects. According to this model, the antitumor effects steeply decreased as the source-cell distance increased when cells of both cell lines were irradiated with 5 Gy without heat. When WiDr cells, a more resistant cell line to radiation and heat, were simultaneously irradiated and heated for 30 min at 44 °C, the effects decreased much less steeply as the distance increased. For A549 cells, a more sensitive cell line, irradiation with hyperthermia even at 42 °C made the decrease in the effects smaller. The largest antitumor effects can be expected at 5–10 mm beneath the esophageal mucosa, where the endoesophageal coil can heat tissues most effectively. SITB can induce larger antitumor effects than brachytherapy alone, especially in submucosal disease, which would favor treatment of advanced cancer.

Key words: Human cancer cell — Radiation therapy — Hyperthermia — Enhancement of radiation effect — Brachytherapy

Combined treatments with radiation therapy and hyperthermia can afford good antitumor effects when applied simultaneously.1) Intraluminal administration of the two modalities is effective. Recently, a balloon applicator initially designed for endoesophageal hyperthermia for esophageal cancer2) was remodeled for simultaneous administration of intraluminal brachytherapy and hyperthermia, and became commercially available in Japan.3) Brachytherapy is administered by a small radioactive source, 192iridium (Ir-192), stepping through a catheter in the esophagus, and hyperthermia is applied by an endoesophageal coil attached to the applicator. Such intraluminal combined therapy should also be applicable to cancers of other luminal organs such as the bronchus and rectum.

Using this applicator, a multi-institutional clinical study on simultaneous intraluminal brachytherapy and hyperthermia for an advanced esophageal cancer was conducted in Japan.4) Hyperthermia is known to enhance radiation effects.1, 5–7) In addition, the endoesophageal coil can most effectively heat the tissue approximately 10 mm outside the surface of the balloon applicator, or beneath the mucosa, where the radiation dose of the brachytherapy steeply decreases. Based on the spatial characteristics of the two modalities, increased antitumor effects can be expected theoretically, especially for submucosal diseases. However, little is yet known, experimentally or clinically, about the spatial distribution of antitumor effects.

We have developed an in vitro treatment model to simulate clinical simultaneous intraluminal thermobrachytherapy (SITB). Using this model, we estimated the spatial distribution of antitumor effects of the combined treatment around the esophagus by directly extrapolating the findings of (i) radiation/heat treatments of two human cancer cell lines in the present study and (ii) the spatial distribution of temperatures achievable with the endoesophageal coil.3)

MATERIALS AND METHODS

Cell lines Two human cancer cell lines were used in the present study: (1) a colon cancer cell line (WiDr) and (2) a lung cancer cell line (A549). The plating efficiencies of these cells were 40.4% (95% confidence interval: 36.9–44.0%) for WiDr cells and 40.9% (37.8–44.1%) for A549 cells.

Radiation source MicroSelectron-HDR with a single source of iridium-192 (Ir-192, Nucletron International, B.V., Veenendaal, The Netherlands) was used. The source strength decays with a half-life of 72 days, and the mean
radioactivity of the source used was 218 GBq (205–232 GBq).

**Brachytherapy** A single straight cylindrical target volume (a planned volume to which a prescribed dose was administered) 9 cm in length and 2 cm in diameter was chosen and a catheter through which the source stepped was set in the center of the target volume. Dose reference points were set 5 mm equidistant along the catheter at 10 mm distance from the catheter. Radiation doses were calculated at these points. The step length of the radioactive source was 2.5 mm. For the treatment planning, PLATO-BPS v. 13.1 (Nucletron International, B.V.) was used to make a flat isodose surface to cover the target volume. Dwell times of the source in each dwell position were optimized by the dose-point optimization method (mode: on dose-points only).8)

**Hyperthermbrachytherapy** Logarithmically growing cells were inoculated into eight wells in a row on a 96-well plate (Corning, New York, NY) at 22 h before treatment. Each well contained 100 to 1600 cells in RPMI medium (200 µl) according to radiation dose. The plate was sealed and immersed in the water bath (Thermobath T-10, Thermonics, Tokyo) to heat cells for 30 min. The plate was set 10 mm above the catheter to make the source-cell distance 10 mm. In the middle of the 30-min heat application, the Ir-192 source stepped through the catheter and irradiated the cells in the wells. When heating finished, the plate was removed from the bath, and incubated in 95% air/5% CO2 at 37°C for seven days (WiDr) or five days (A549). Then the cells were fixed and stained with crystal violet in methanol, and colonies consisting of more than 50 cells were counted. One plate was prepared as a control in each experiment and, in addition, another plate was heated but not irradiated as a “0 Gy” plate.

**Data analysis** For heat alone experiments, dose-response curves were drawn and $D_0$, or the reciprocal of the slope of the curve was calculated. For experiments involving combined treatments, dose-response curves were drawn and 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. Regression analysis to estimate $D_0$ and $D1$ was performed using StatView v.4.5J (Abacus Concepts, Berkeley, CA). The equation used to obtain a 95% confidence interval of $D1$ was described previously.9) Mann-Whitney’s $U$ test was used to compare surviving fractions.

**Simulation of SITB** The conditions of simulated treatment were that the applicator was assumed to be inserted in the center of the esophageal lumen, and the balloon of the applicator dilated 20 mm in diameter. A circumferential esophageal tumor 30 mm in thickness and 80 mm in longitudinal length was then heated for 30 min with the endoesophageal coil at various temperatures. Endoesophageal brachytherapy was administered in the middle of the hyperthermia, and the prescribed radiation dose was 5 Gy at 15 mm from the source axis, or at 5 mm beneath the mucosa. Data on the spatial distribution of temperatures achievable with the applicator were based on the study of Fuwa et al.9) The surviving fraction was calculated with three patterns of temperature distribution: i) Temp44. The temperature in tumor ranged from 42 to 44°C (original data from Fuwa et al., open circle in Fig. 4). ii) Temp43. The tumor temperature ranged between 41–43°C (open square), and iii) Temp42. 40–42°C (arrowhead). Radiation doses and temperatures varied according to the distances from the endoesophageal applicator. Distribution of surviving fractions on the mid-plane (perpendicular to the axis of the esophagus) of the applicator was calculated.

**RESULTS**

**Heat alone treatments** Fig. 1 shows the heat sensitivity of the two cell lines. Cytotoxic effects of hyperthermia were noted at the temperatures of 43–45°C for WiDr cells

![Fig. 1. Dose-response curves for two cell lines heated at various temperatures. Bars are mean±SE. □ 40°C, △ 41°C, ● 42°C, × 43°C, ■ 44°C, ○ 45°C.]
and 42–45°C for A549 cells. $D_0$ values at each temperature are shown in Table I.

### Simultaneous combined treatment with radiation and hyperthermia

Dose-response curves for the simultaneous radiation and heat treatments of the two cell lines are shown in Fig. 2. The surviving fractions for WiDr cells after 8 Gy irradiation significantly decreased when the cells were irradiated at the comparatively low temperature of 42°C, the temperature at which heat itself showed little antitumor effect, compared with the irradiation at 37°C ($P<0.05$). Irradiation at 40 and 41°C showed no significant change in the surviving fractions. For A549 cells, irradiation at 41°C or lower showed no significant change compared with irradiation at 37°C. The values of $\alpha$, $\beta$, and $D_1$ at each temperature are summarized in Table II. The $\beta$ values at 43°C and 44°C were significantly larger than that at 37°C for WiDr, and the $\alpha$ value at 44°C was significantly larger than those at 37°C and 40–42°C for A549.

### Sequence of hyperthermia and radiation

The dose-response curves when the cells were sequentially treated with irradiation and heat are shown in Fig. 3. In both cell lines, the cells showed the least surviving fractions when they were irradiated and heated simultaneously, compared with cells for which the two treatments were sequentially administered. However, the differences in $D_1$ values in the dose-response curves were not significant.

### Assumed antitumor effects in simulated treatment of SITB

Surviving fractions were plotted against the distance from the radioactive source (Fig. 4). For both cell lines, when the cells were irradiated at 37°C, surviving fractions steeply decreased as the distance from the source increased. For WiDr cells, a radio- and heat-resistant cell line, the curve of surviving fractions with the temperature distribution of Temp44 decreased, and the lowest surviving fraction was observed at 15 mm from the source, or 5 mm beneath the esophageal mucosa. With Temp43 and Temp42, the curves were similar to that in the non-heat experiment. In contrast, for A549 cells, a sensitive cell line to both radiation and heat, the curve, even with Temp42, was shifted downwards compared with that of the non-heat experiment, and made the antitumor effect curve a less steeply decreasing one. The surviving fractions decreased more with Temp43, and the curve fell further to the level of surviving fraction of approximately 0.001 with Temp44. The largest effects were noted at 15–20 mm from the source, or 5–10 mm beneath the mucosa.

### DISCUSSION

To perform simultaneous hyperthermo-radiotherapy effectively for cancers of luminal organs such as the esophagus, bronchus, and rectum, intraluminal administration of radiation therapy (brachytherapy) and hyperthermia is a favored mode of treatment. Using this technique, a clinical study on SITB for advanced esophageal cancer was conducted at eight institutes in Japan. In this study, a total dose of 60 Gy is typically administered first by external beam irradiation, and then intraluminal brachytherapy and hyperthermia are simultaneously administered as a boost. The dose of brachytherapy is 6–8 Gy in two weekly fractions of 3–4 Gy each at 5 mm beneath the esophageal mucosa, or 15 mm from the source axis (because the balloon of the applicator was dilated to 20

### Table I. $D_0$ Values for Heat Alone Treatment

| Temp. (°C) | WiDr | A549 |
|------------|------|------|
| 40         | 20   | 12   |
| 40–63      | (12–63) | (4.7– ) |
| 41         | 13   | 23   |
| 8.5–24.0   | (13–111) | (13–111) |
| 42         | 8.2  | 0.94 |
| 6.5–11.0   | (0.82–1.10) | (0.82–1.10) |
| 43         | 0.59 | 0.35 |
| 0.48–0.79  | (0.29–0.43) | (0.29–0.43) |
| 44         | 0.097 | 0.11 |
| 0.081–0.12 | (0.095–0.12) | (0.095–0.12) |
| 45         | 0.07 | 0.058 |
| 0.061–0.083 | (0.050–0.069) | (0.050–0.069) |

Mean±2 SE.
Intraluminal Thermobrachytherapy

The hyperthermia is designed to provide a temperature of 42.5°C on the surface of the balloon for 30 min. Clinical data on the treatment are being accumulated.

In the present study, we confirmed that the cytotoxic effects of radiation increased when it was combined with hyperthermia at 42–43°C or higher. The increase in the effects was most apparent when the irradiation and heat were simultaneously administered. These findings agree with those of Overgaard.1) The dose-response curves for the combined treatment moved downward to parallel the non-heated curve as the temperature increased. Moreover, some of the slopes of the curves became slightly steeper at higher temperatures (Fig. 2). When WiDr cells were irradiated at 42°C, a temperature at which heat itself showed little antitumor effect, the surviving fraction was also significantly decreased compared with that at 37°C. This phenomenon is known as the “mild temperature hyperthermia effect.” This was not observed for A549 cells.

The effect of mild temperature hyperthermia is typically observed when low-dose-rate irradiation (LDRI) is combined with hyperthermia.6, 7, 10) This is because cellular repair of radiation damage is inhibited by hyperthermia.5) In contrast, when high-dose-rate irradiation (HDRI) is combined with hyperthermia, the enhancement of the radiation effect has been shown to be much smaller than in hyperthermia combined with LDRI.6, 7, 10) This is explained by the finding that recovery from radiation damage takes place to a lesser extent in HDRI compared with the repair in LDRI.5)

Thus, the simultaneous combination of HDRI and hyperthermia can radiobiologically induce only a small enhancement of the radiation effect. From the clinical viewpoint, however, increased effects, greater than a simple additive effect, can be expected with SITB. Firstly, as shown in Fig. 4, the antitumor effect steeply decreased as the distance from the source increased when radiation alone was administered. In contrast, when radiation and

Table II. α, β and D1 for Two Cell Lines Simultaneously Irradiated and Heated

| Cell | Temp. (°C) | α<sup>a</sup> | β<sup>a</sup> | D1<sup>b</sup> |
|------|------------|---------------|---------------|---------------|
| WiDr | 37         | 0.285         | 0.04          | 7.14          |
|      | 40         | 0.15          | 0.062         | 7.17          |
|      | 41         | 0.209         | 0.08          | 6.11          |
|      | 42         | 0.004         | 0.115         | 6.07          |
|      | 43         | −0.295        | 0.309         | 4.28          |
|      | 44         | 0.33          | 0.268         | 2.61          |
|      | 45         | −1.321        | 1.65          | 1.11          |
|      | 46         | −3.741        | (0.106–3.194) | (−1.95)       |
| A549 | 37         | 0.329         | 0.081         | 5.73          |
|      | 40         | 0.458         | 0.067         | 5.56          |
|      | 41         | 0.248         | 0.056         | 7.05          |
|      | 42         | 0.466         | 0.173         | 3.89          |
|      | 43         | 0.851         | 0.092         | 2.81          |
|      | 44         | 2.3311        | −0.283        | 0.22          |
|      | 45         | 1.177–3.485   | (−0.677–0.111) | (−1.15)       |

<sup>a</sup) Mean±2 SE.
<sup>b</sup) Mean with 95% confidence interval (CI).

Blanks in parentheses show that the lower bounds of the 95% CI are very small, suggesting that the estimated CIs of D1 levels in these temperatures statistically have little meaning.
hyperthermia were simultaneously combined, the effect of hyperthermoradiotherapy decreased much less steeply for WiDr cells heated to 44°C and for A549 cells heated to 42–44°C. This is because the deeper parts of a ‘thick’ esophageal tumor could be heated at higher temperatures than the superficial part of the tumor, and consequently the submucosal disease would be subject to greater antitumor effects by hyperthermia than the mucosal disease. This favors the treatment of locally advanced cancers that often present a bulky mass. For heat-sensitive cell lines, higher temperatures such as 43–44°C might not always be necessary to make the antitumor effect curve a flat one and to avoid hot spots of the antitumor effects, although the heat sensitivity of a tumor is rarely known before treatment in a clinical setting. Secondly, the intraluminal treatment is administered as a boost therapy following a full dose of external beam irradiation. At that time, possible residual tumors consist of comparatively radioresistant cells such as hypoxic cells. The hypoxic tumor is known to be more sensitive to hyperthermia than an oxic tumor. In addition, the microvasculature in and around the tumor is damaged after 60 Gy irradiation (known as tumor bed effect), and this also favors hyperthermia.

Thus, owing not only to the spatial characteristics of the two modalities, but also to the sequence of external beam irradiation and intraluminal thermobrachytherapy, the effect of SITB can be expected to be greater than additive. The present study showed that this combination of radiation and hyperthermia can actually be favorable, especially in its spatial distribution of the antitumor effect.

The two cell lines of A549 and WiDr were used in the present study for the following two reasons. Firstly, to simulate clinical therapy as closely as possible, we established a very complex experimental setting. In this complicated setting, to avoid technical errors, cell lines with which we have abundant experience and data were chosen. Secondly, as stated in the introduction, intraluminal thermobrachytherapy is applicable to cancers of other luminal organs such as the bronchus and rectum (A549 is a lung cancer cell line and WiDr is a colon cancer line).
The present findings were obtained in vitro and with only two cell lines, so care is needed in extrapolation to clinical therapy. Many additional factors such as necrosis, hypoxia, pH, and tumor vascularity are relevant in vivo. However, these findings may be of help to analyze clinical observations.

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