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Biological Cell Survival Mapping for Radiofrequency Intracavitary Hyperthermia Combined with Simultaneous High Dose-rate Intracavitary Irradiation

Hiromasa Kurosaki, Hideyuki Sakurai,[1] Norio Mitsuhashi, Yoshio Tamaki, Tetsuo Akimoto, Takeo Takahashi, Masaya Furuta, Jun-ichi Saitoh, Kazushige Hayakawa and Hideo Niibe

Department of Radiology and Radiation Oncology, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511

We examined the best way to combine recently developed radiofrequency intracavitary hyperthermia with simultaneous high dose-rate intracavitary brachytherapy in an original experimental model. Temperature distribution was measured with an experimental phantom which was immersed in a water bath with the temperature controlled at 37°C. Radiation dose distribution was calculated with a treatment-planning computer. Cell survival was measured by colony assay with HeLa-TG cells *in vitro*. Radiation dose response at 1–7 Gy and time response with hyperthermia in the range of 40–46°C were estimated. Radiation dose-response curves in simultaneous treatment with hyperthermia for 30 min at 37 to 46°C were estimated and the surviving fractions in combined treatment were plotted against temperature. For intracavitary radiation alone, cell survival rates increased with increasing distance from the source. For intracavitary hyperthermia alone, the maximum temperature was observed at a depth of 13 mm from the surface of the applicator under suitable treatment conditions. Homogeneous cell killing from the surface of the applicator to a tumor depth of 13 mm was observed under a specific treatment condition. Our experimental model is useful for evaluating the best simultaneous combined treatment.

Key words: Intracavitary hyperthermia — Intracavitary brachytherapy — High dose-rate irradiation — Radiofrequency hyperthermia — Experimental study

The most effective combination of radiation and hyperthermia is considered to be simultaneous exposure, rather than sequential treatment.[1, 2] However, external hyperthermia combined with simultaneous external irradiation for deep-seated tumors is difficult in clinical practice because it requires special devices.[3–5] When simultaneous treatment can be applied to deep-seated tumors, an intracavitary or interstitial approach is probably necessary. A combination of recent developed radiofrequency (RF) intracavitary hyperthermia and intracavitary high dose-rate irradiation with iridium-192 sources seems to be a good method for simultaneous exposure to treat deep-seated advanced tumors.

Although simultaneous treatment is theoretically attractive, quantitative data on temperature distribution under various heating conditions and on cytotoxicity, including hyperthermic radiosensitization, are not available. The aims of this study were therefore to collect temperature data on radiofrequency intracavitary hyperthermia with an original phantom under various heating conditions and to obtain cell survival maps in the case of simultaneous treatment with an *in vitro* experimental system. Finally, we investigated the conditions giving the most uniform cytotoxic effect, which should be the best for combined intracavitary hyperthermia and intracavitary radiation treatment.

[1] To whom correspondence should be addressed.
E-mail: hsakurai@med.gunma-u.ac.jp

MATERIALS AND METHODS

Temperature measurement in RF intracavitary hyperthermia A phantom was used for measuring temperature distribution (Fig. 1). An Endoradiotherm 100A (Olympus, Tokyo) operating at 13.56 MHz was selected as a device for intracavitary hyperthermia. In this system, the temperature of running water within the applicator can be changed. The temperature at a couple of centers on the applicator surface was controlled by an auto-regulatory power on-off system. In the center of the phantom, an ES-15 applicator, which is an internal applicator 100 mm long and 15 mm in diameter, was placed. The cylindrical agar phantom consisted of 4.00% agar, 0.24% sodium chloride, 0.10% sodium azide and 95.66% water. The agar phantom was cooled by external circulating water at a controlled temperature of 37°C. For temperature measurement, multipoint thermocouple thermometers attached to a Thermotron-RF8 (Yamamoto Vinita Co., Ltd., Osaka) were inserted into the agar phantom and temperature at a total of 42 points was measured. The RF power was kept at 100 W. Approximately 30 min after the start of heating, the temperature of the agar phantom reached equilibrium with the external circulating water at 37°C. The heating conditions were as follows: the temperature on the surface of the internal applicator was maintained at 40 or 42°C, and the temperature of running water within the internal applicator was kept at 37 or 40°C. Hyperthermic treatment con-

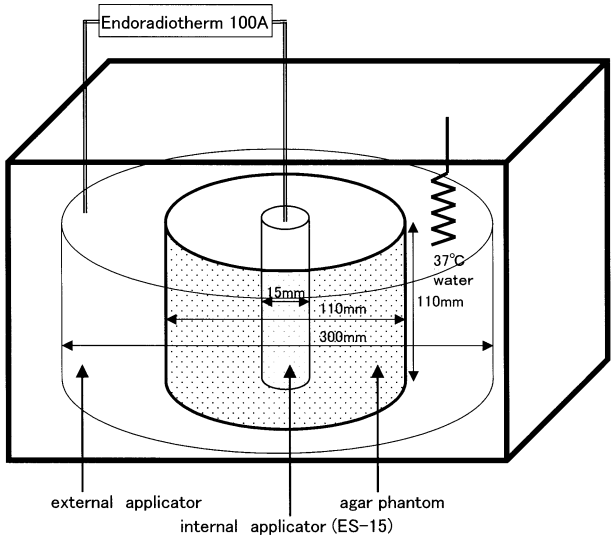

Fig. 1. Schematic diagram of dynamic phantom for measurement of temperature distribution under various heating conditions. An internal applicator (ES-15) was placed in the center of the agar phantom. The phantom was cooled by outer circulating water at 37°C.

ditions are defined in this manuscript as follows: when the temperature at a couple of centers on the applicator surface was kept at 40°C and the temperature of running water within the internal applicator was controlled at 37°C, the treatment condition was termed T40-W37. Measurements of temperature were independently performed 4 times and the average was used for subsequent data analysis.

Radiation dose distribution in intracavitary brachytherapy Radiation dose distribution was calculated with a computer, PLATO, attached to a Microselectron-HDR (Nucletron, Veenendaal, Holland). A linear arrangement with a length of 100 mm was simulated with an iridium-192 point source. Dose points were arranged at a depth of 12.5 mm from each source point, which was equal to 5 mm depth from the surface of the ES-15 applicator. After the doses for dose points were normalized, optimization was performed by means of a dose point optimization program on PLATO. Radiation dose distributions of 3, 4 and 5 Gy at the dose points were calculated.

Cell culture and survival measurement A human cervical adenocarcinoma cell line, HeLa-TG cells, was kindly provided by the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University. The cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum, antibiotics and 0.3% HEPES (Immunobiological Laboratories Ltd., Fujioka, Gunma). Cells growing exponentially with an apparent doubling time of 31 h were used in this study. Cell suspensions were prepared from stock culture flasks. The cell survivals were estimated by means of the Courteney soft agar clonogenic assay. An appropriate number of cells in agar was plated into the tubes. The final agar concentration was 0.3%, and the volume of the agar plugs was 1 ml per tube. The cells in the agar were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air for 16 h prior to the treatment procedures. After the treatments, the cells in the agar were incubated for 14 days to permit colony formation before being fixed with 6% glutaraldehyde. The fixed colonies were crushed between two thin glass plates and counted under a microscope. Colonies containing more than 50 cells were counted as survivors. Surviving fractions were calculated by comparison with the control tubes. Each data point was derived from the results of at least three independent experiments. The plating efficiency was 89.2±3.9% (n=17).

Treatment method for cells Hyperthermia at 40 to 46°C was applied to the cells by immersing the sealed Falcon 2054 tubes containing the agar plugs in a circulating water bath in which the temperature was controlled to within 0.05°C (Julabo, Allentown, PA). The agar temperature reached the intended value within 3 min. Cell survival curves for hyperthermia were estimated.

Radiation treatment of the tubes was done with an iridium-192 source attached to a Microselectron-HDR. A radiation treatment device was designed with PLATO. The sources were arranged cylindrically (80 mm in diameter) and the cells in the agar were placed exactly at the center of the circle (Fig. 2). Under this condition, the central dose on the plane across the sources was substantially uniform. The overall dose-rate was more than 0.50 Gy/min. Radiation dose-response curves were estimated in the range from 1 to 7 Gy at a temperature of 37°C, controlled by the water bath.

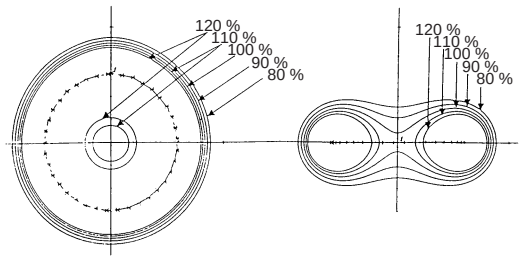

Fig. 2. The radiation dose distribution calculated with the treatment-planning computer. The cells in agar were placed at the center of the circle. The overall dose-rate was above 0.50 Gy/min.

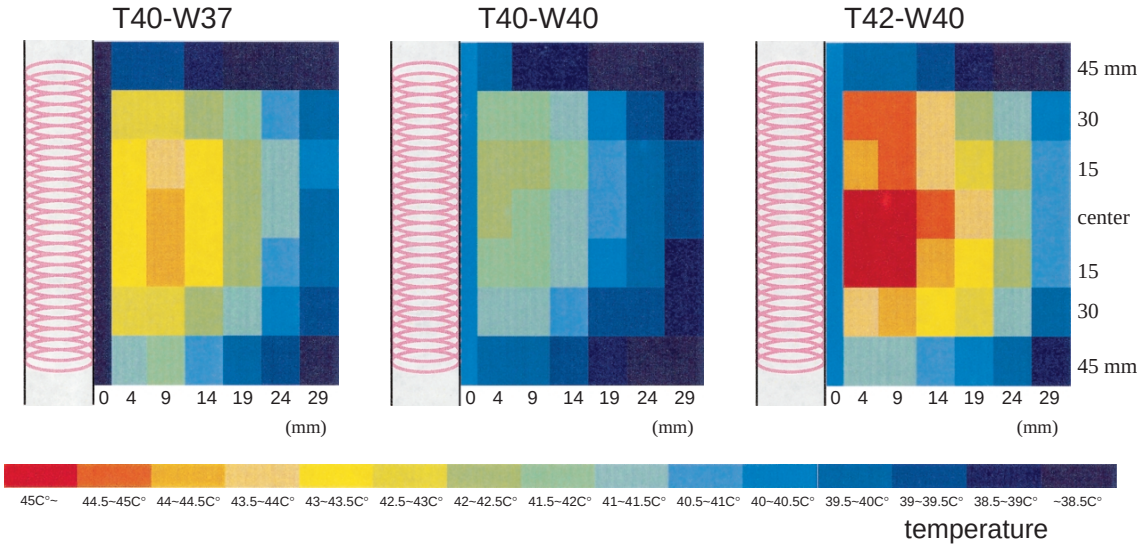

Fig. 3. The temperature distribution maps for several heating conditions. Each column shows the temperature measurement point. The colors of each column indicate measured temperature. Hyperthermic treatment conditions are symbolized. When temperature on the applicator surface is controlled at 40°C and the temperature of the running water within the internal applicator is controlled at 37°C, the treatment condition is symbolized as T40-W37.

For the treatment protocol of simultaneous hyperthermia and radiation, the duration of hyperthermia was 30 min. Radiation therapy started 10 min after the start of heating. For simultaneous treatment, the irradiation device was directly immersed in the water bath. Radiation dose-response curves in the case of simultaneous heating for 30 min were estimated.

Data analysis and cell survival mapping The data points on the survival curves were fitted to a linear equation with the Statview program version 4.0 (Abacus Concept, Inc., Cary, NC) on a Macintosh computer. The D_0 values were calculated as the reciprocal of the slope of the exponential region of the radiation dose-response curves. Similarly, the T_0 values were calculated as the reciprocal of the slope of the time-response curves for hyperthermic treatment. By these methods, all curves were approximated to linear equations.

For hyperthermia alone, radiation alone and simultaneous hyperthermia plus radiation, the cell surviving fractions at the foregoing 42 temperature measurement points were calculated with the linear equations. The cell surviving fractions are shown as 16 kinds of color in the figures in this manuscript.

RESULTS

Temperature distribution for heat alone Fig. 3 shows the temperature distribution of RF intracavitary hyperthermia under various conditions. The phantom was substantially heated between depths of 4 and 14 mm. In the longitudinal direction, temperatures at 30 mm from the center of the phantom were elevated. The maximum temperature was observed at a depth of 9 mm across the center of the phantom. The patterns of temperature

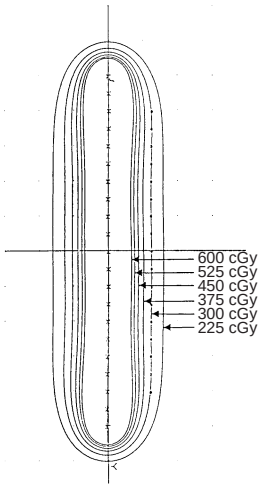

Fig. 4. Calculated radiation dose distribution of 3 Gy at the reference points for intracavitary brachytherapy. The linear arrangement with a length of 100 mm is simulated by using an iridium-192 point source. Dose points are arranged at 12.5 mm depth from each source point. The doses were normalized, then optimized.

distribution varied according to treatment conditions, there being a slight increase in T40-W40, and a remarkable increase with a hot spot above 45°C in T42-W40.

Radiation dose distribution A case of dose distribution in intracavitary HDR brachytherapy is shown in Fig. 4. Dose points were arranged at a depth of 12.5 mm from each source point. Since the dose was reduced with increasing distance from the source, it follows that the absolute dose decreased with increase in the depth of the applicator.

Cell survival for hyperthermia alone The response of HeLa TG cells to various hyperthermic temperatures alone is shown in Fig. 5. No cell killing was observed at 40°C and only 40% of the cells was killed during 8 h at 41°C. Above 42°C, higher temperature resulted in more cell killing. The development of chronic thermotolerance was observed at 42 and 43°C.

Cell survival for radiation alone and for simultaneous treatment Fig. 6 shows the cellular response to radiation and to various degrees of simultaneous hyperthermia for 30 min plus radiation. From 41 to 45°C, the cell kill was normalized with respect to that due to hyperthermia alone. The pattern of modification due to hyperthermia was mainly shoulder reduction. Above 42°C, higher temperature produced a greater radiosensitizing effect.

Tumor cell survival mapping The cell survival distribution maps for intracavitary hyperthermia alone for 30 min or intracavitary radiation alone at several dose levels are shown in Fig. 7. In the case of hyperthermia alone for 30 min, very weak effects were observed in T40-W37 and in T40-W40, whereas a significant cytotoxic effect was observed with the highest temperature setting of T42-E40. As shown in Fig. 4, the surviving fractions increased with decrease in the radiation dose. The maximum cell killing effect was observed on the surface of the applicator. The survival maps for the combination of hyperthermia with radiation are shown in Fig. 8. For the hyperthermic treatment with T40-W40, the pattern of the survival maps was similar to that of radiation alone, because the effect of hyperthermia was relatively less. Conversely, for T42-W40, the pattern of the survival maps was similar to that of heat alone because the effect of hyperthermia was too large. Finally at T40-W37, relatively homogeneous cell killing effects over a wide area were observed, combined with an intracavitary radiation dose of 3 Gy.

DISCUSSION

A number of investigators have shown that measured temperature and tumor heating time correlate well with tumor response.[6–9] The problems have been how to effectively deliver heat to deep-seated tumors and how to con-

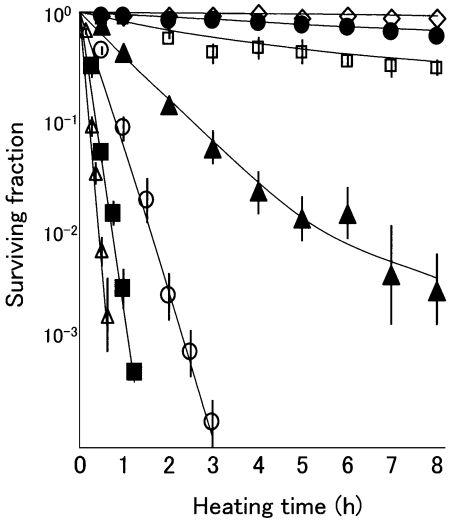

Fig. 5. Time-response curves of HeLa-TG cells to hyperthermia at 40 to 46°C. Bars show ±2 standard errors, where these exceed the size of the symbol. ◇ 40°C, ● 41°C, □ 42°C, ▲ 43°C, ○ 44°C, ■ 45°C, △ 46°C.

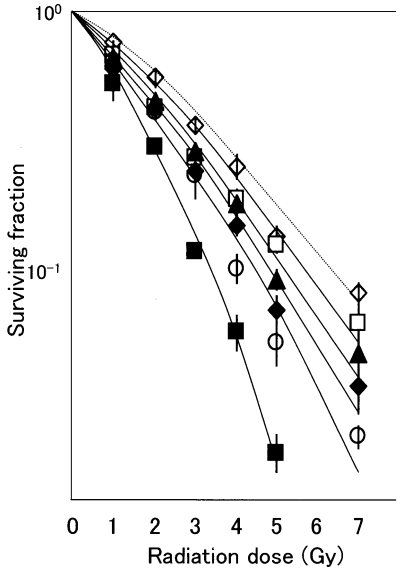

Fig. 6. The response of HeLa-TG cells to radiation and to various degrees of hyperthermia for 30 min simultaneously with radiation. The dashed line represents the survival curve for radiation alone (i.e., radiation at 37°C). The results for simultaneous combined treatments were normalized to those for cell kill due to heat alone for 30 min. The bars show ±2 standard errors, where these exceed the size of the symbol. D_0 values were calculated from the slopes of the curves shown above. ◇ 37°C, □ 42°C, ▲ 43°C, ◆ 43.5°C, ○ 44°C, ■ 45°C.

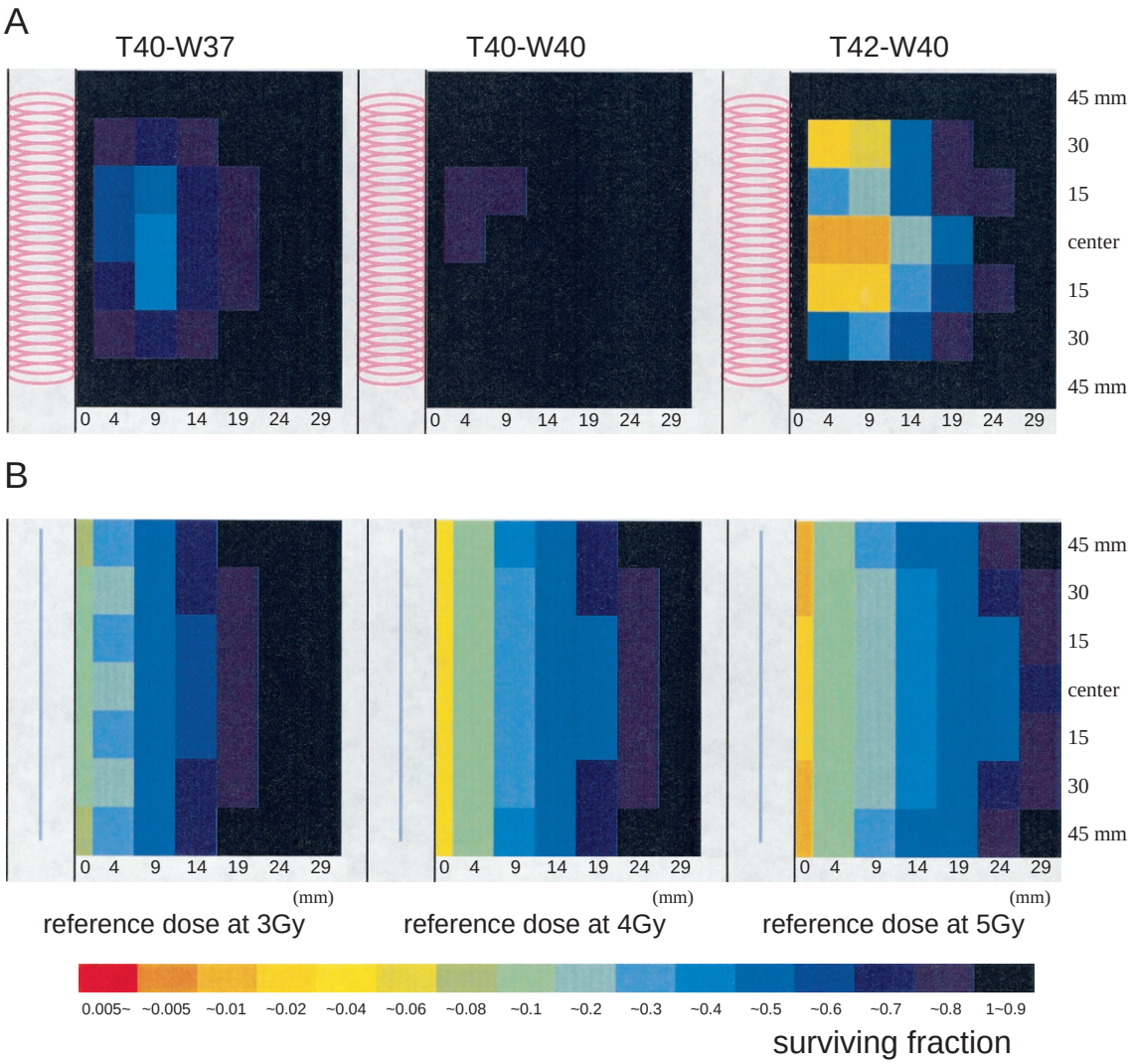

Fig. 7. (A) Maps of cell survival distribution due to intracavitary hyperthermia alone for 30 min. (B) Maps of cell survival distribution due to intracavitary radiation alone at several dose levels. Each column shows cell survival levels at the measurement point. The colors indicate the calculate surviving fractions.

tinuously measure the temperature in these deep-seated tumors during hyperthermia.[10–13] There are certain problems involved in invasive measurement of intratumoral temperature, especially in deep-seated tumors. In esophageal cancer and rectal cancer, for instance, it is very difficult to insert the catheter into the tumor. Although some researchers have demonstrated that temperatures can be measured under RF or microwave fields without perturbation,[14, 15] only a few point temperatures were measured. Non-invasive thermal dosimetry seems promising,[16] but is not readily available. Unfortunately, the tumor temperature can only be determined by an invasive procedure. In general, the RF intracavitary hyperthermic device should be controlled by the temperature setting on the surface of the applicator, which is considered to be the same as that on the tumor surface, but the temperature on the surface of the tumor is not exactly the same as the temperature within the tumor. Even though thermal parameters of temperature on the surface of the tumor have been reported to correlate with tumor response in intracavitary hyperthermia,[17] it is highly desirable that the true temperature distribution be determined. In this study, we measured temperature distributions under several heating conditions by using a dynamic phantom. Our observations indicate

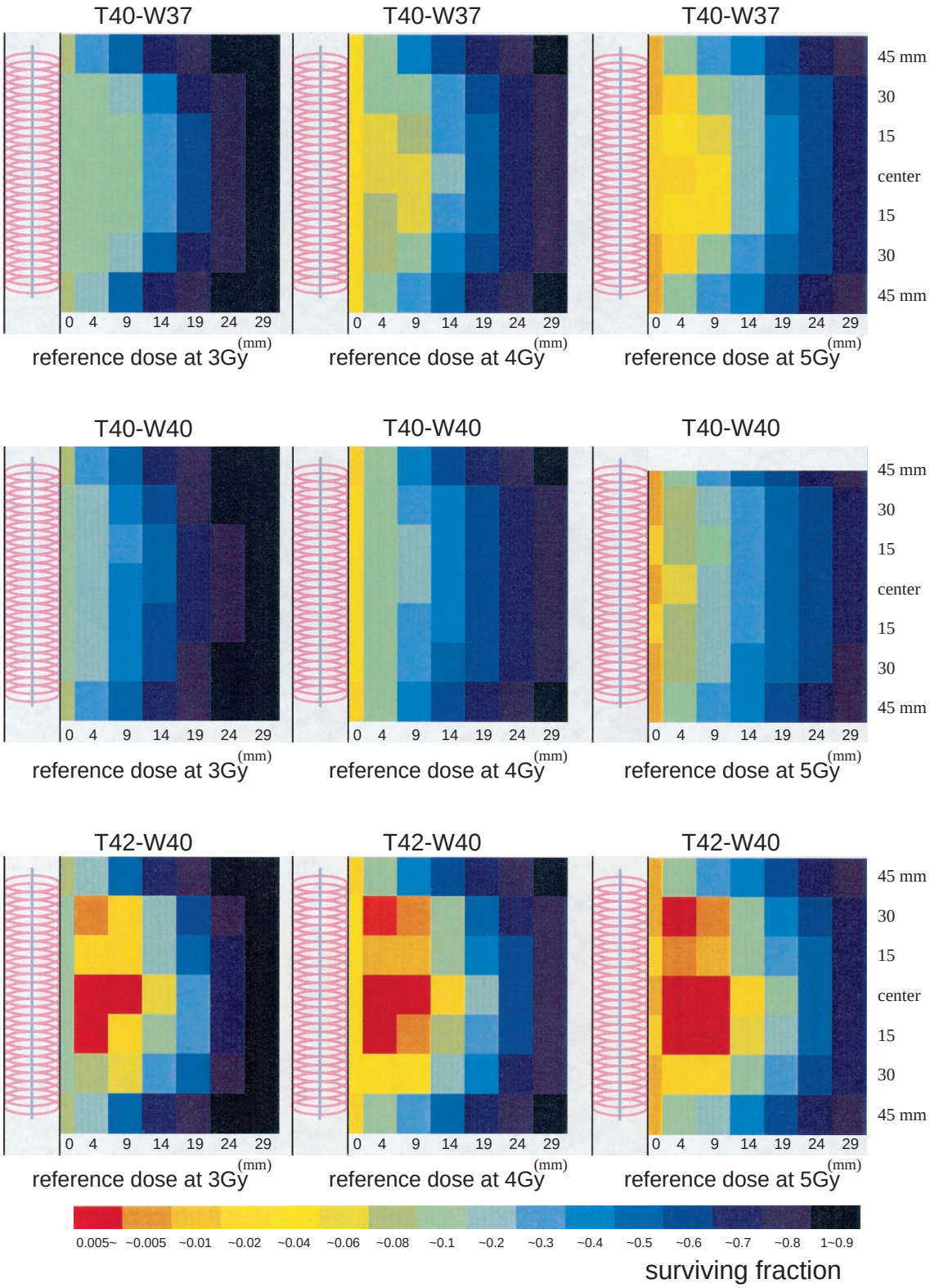

Fig. 8. Maps of cell survival for combinations of hyperthermia and simultaneous radiation. Each column shows cell survival levels at the measurement point. The colors are shown as calculated surviving fractions.

that a higher temperature setting, T42-W40, did not provide a homogeneous temperature distribution. This heating condition will result in an inhomogeneous cytotoxic effect on the tumor tissue. On the other hand, in the case of a lower temperature setting, T40-W40, the temperature increase was insufficient.

Hyperthermia is known to biologically enhance the effect of ionizing radiation. The two were reported to have the strongest anti-tumor effect when applied simultaneously.[1, 2, 10] The radiosensitizing effect decreases rapidly as a function of the time interval between the two therapies. Ryu *et al.* recently reported an animal model, which was treated with a combination of irradiation and simultaneous hyperthermia, and concluded that significant tumor growth delay was observed with the addition of hyperthermia delivered simultaneously, without any adverse side effect.[18] The simultaneous combination is an attractive way to increase tumor response, but also causes greater injury to normal tissue than sequential application. Overgaard reported that thermal enhancement ratios (TERs) were increased 2.5 times in both tumor and normal tissue in his animal model.[1] Because the therapeutic gain factor (TGF) of simultaneous combination, which is the ratio of TER of the tumor tissue to TER of the normal tissue, was decreased in comparison with sequential treatment,[1, 10] selective tumor heating was necessary for simultaneous treatment. Intracavitary hyperthermia may be a better way to selectively heat the tumor than external hyperthermia. Various applicators and application techniques for the clinical application of intracavitary hyperthermia have been reported[19–24] and it was concluded that intracavitary hyperthermia could improve local control.

Even when both the exact temperature and radiation dose applied to a specific point can be estimated, the cytotoxic effect of simultaneous treatment cannot be precisely estimated, because, in principle, the effect of simultaneous treatment involves three different mechanism, namely radiation cytotoxicity, direct hyperthermic cytotoxicity and hyperthermic radiosensitization. We therefore need to have quantitative cell killing data in simultaneous treatment. In this study, we directly estimated the cytotoxic effect of combined treatment with an *in vitro* experimental model, and then we plotted the biological data on radiation-temperature distribution maps. This study has provided important information prior to clinical application of this attractive method.

From the calculation of radiation dose distribution, an intraluminal superficial tumor attached to the applicator surface can be substantially cured with intracavitary brachytherapy alone, but the dose distribution of intracavitary brachytherapy is unsuitable for treating a large-volume tumor. When using two different methods, there is the possibility that a combination of an inhomogeneous radiation dose and inhomogeneous temperature distribution will have a homogeneous cell killing effect on the tumor tissue. In this study, we demonstrated that a hyperthermic temperature setting of T40-W37 simultaneously combined with an intracavitary radiation dose of 3 Gy was the treatment of choice; a higher setting could cause injury to normal tissue, and a lower temperature setting might yield no additional effect.

In conclusion, our experimental model is useful for estimating the optimum combination for simultaneous intracavitary treatment. It should provide data for clearly establishing both the safety and effectiveness of the simultaneous combination.

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