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Radiation, Hyperthermia, and Their Combination in Treatment of Chemically Induced Autochthonous Tumors in Mice

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All autochthonous tumors induced chemically in the thighs of C3H/He mice showed recurrence after single X-irradiation with 20–60 Gy when they were 10 mm in diameter, although this treatment caused temporary, dose-dependent regression. Hyperthermia for 30 min at 43.0° alone had little effect. However, in 2 of 16 mice, hyperthermia after irradiation at 60 Gy resulted in complete cure, i.e., survival of mice without recurrence for more than 120 days after treatment. These results indicate that the combined treatment of radiation and hyperthermia is necessary to obtain the cure of mouse autochthonous tumors.

Key words: Autochthonous tumor — Hyperthermia — Radiation therapy — Mouse

Experimental tests on therapy of autochthonous tumors are urgently needed for developing realistic therapeutic treatments of human tumors, irrespective of whether the treatment is surgery, chemo- or immunotherapy, radiation, or hyperthermia. Hyperthermia has been widely accepted as a new treatment of cancer,[1] and has been used clinically for therapy of various surface and deep-seated human tumors.[2] The effectiveness of hyperthermia has been studied using cultured tumor cells or transplantable tumors.[3] However, the response of transplantable mouse tumors to hyperthermia is quite different from that of human tumors: transplantable mouse tumors are more sensitive to moderate heating, and their local recurrence is less frequent.[4] Spontaneous or autochthonous tumors provide a more realistic experimental model for use in testing therapeutic treatments. But, because of technical difficulties and the low tolerance to heat of small animals, most experiments on the effect of hyperthermia on spontaneous tumors have been carried out in relatively large animals, such as cats and dogs.[5, 6]

In the present study, we examined the therapeutic values of radiation, hyperthermia, and their combination on chemically induced autochthonous tumors in mice, and found that combined treatment with radiation and hyperthermia was significantly, though not highly, effective.

Abbreviations used: MCA, 3-methylcholanthrene; TGT, tumor growth time.

MATERIALS AND METHODS

Animals Female C3H/He mice (Charles River Japan, Kanagawa-ken), were used for experiments at 6 weeks of age. The mice were kept five or six to a cage under specific pathogen-free conditions, and were provided with a laboratory diet (CE-2, Clea Japan, Tokyo) and sterilized water *ad libitum* under the guidelines of the National Cancer Center Research Institute. Groups of five to sixteen mice were used in experiments.

Induction of Autochthonous Tumors A sterilized solution of 0.5 mg/ml of MCA (Spectrum Chemical Mfg. Corp., Redondo Beach, CA) in olive oil was injected sc into the thigh of mice through a 26 G needle, as we described elsewhere.[7] Ninety percent of the tumors produced in the groin of female ICR mice by this method were identified histologically as fibrosarcoma.[8]

Irradiation Single X-irradiation (20, 40, or 60 Gy) was performed when the autochthonous tumors became approximately 10 mm in diameter. The mice were lightly anesthetized with Nembutal (Abbott Laboratories, North Chicago, IL), and the tumor on their thigh was locally irradiated with 250 kVp X-rays at a dose rate of 3.3 Gy/min generated by a Maxitron 300 (General Electric Co., Milwaukee, WI), and filtered through a 2 mm Cu filter with collimation of 25×20 mm^2. The dose rate was measured with an ionization chamber (Ionex, type 2500/3; Nuclear Enterprises, Edinburgh, UK) and a Fricke dosimeter.

Hyperthermia Immediately after irradiation, the mouse thigh with the tumor was immersed in a circulating water bath at 43.0° for 30 min. The temperature at the center of the tumors was confirmed to be about 43.0° by measurement with a thermocouple (Baily Instrument Co., NJ).

Tumor Measurement The size of tumors was measured with a slide caliper at least three times a week, and the tumor volume was calculated as $ab^2/2$, where a and b are the lengths of the long and short axes of the tumor. Relative tumor volumes, calculated as the volume of tumor after treatment as a percentage of the original tumor volume before treatment, were plotted against the time after treatment. The tumor growth time (TGT), i.e., the time required for the tumor volume to reach 5 times that before treatment, was estimated from the growth curve for each tumor. Mice that showed complete tumor regression after treatment and survived more than 120 days without local recurrence or metastasis were considered to be cured. Mice were autopsied and their lungs were carefully examined macroscopically for metastases.

Results

Tumor Induction Tumors developed in all C3H/He mice 70–120 days after subcutaneous injection of MCA into their thigh. This induction rate was much higher than that in ICR mice (88%) reported previously.[7] The average TGT was 15.2 days, as estimated from the tumor growth curves (Fig. 1). The volume doubling time of untreated tumors after reaching 10 mm in diameter was 4.2 days.

Tumor Response to Hyperthermia The temperature at the center of the tumor, monitored by a thermocouple, reached 43.0° within 10 min. The central temperature is expected to be close to the peripheral tumor temperature (within 0.2°) in the anesthetized state.[9] The growth curves of the autochthonous tumors after a single hyperthermic treatment (43.0°, 30 min) are shown in Fig. 1. Hyperthermia alone seemed to have no effect on tumor growth, but to prolong the survival period of mice after treatment slightly, although not significantly (Table I).

Tumor Response to Radiation The tumor responses to single X-irradiation (20, 40, or 60 Gy) are shown in Fig. 2. The survival periods, TGT and rates of complete response of the treated tumors correlated well with the radiation doses (Table I). The incidence of complete regression of autochthonous tumors was dose-dependent; i.e., complete regression was observed in 0 of 7 mice irradiated at 20 Gy, 3 of 10 mice irradiated at 40 Gy, and 11 of 14 mice irradiated at 60 Gy. However, all the mice showed tumor recurrence, except one that died without recurrence within 120 days after irradiation. Therefore, no cure was obtained in the groups treated by radiation alone.

Tumor Response to Combined Treatment The tumor responses to radiation plus hyperthermia are shown in Fig. 2. Hyperthermia did not have a synergistic effect on the initial

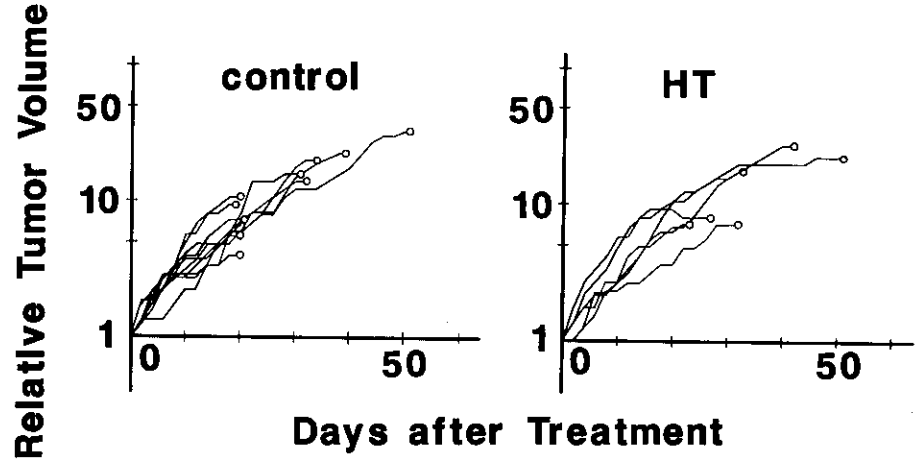

Fig. 1. Growth kinetics of MCA-induced autochthonous tumors in C3H/He mice. Control (left); treated by hyperthermia only (HT) for 43.0° at 30 min (right).

Table I. Summary of Responses and Cures of Autochthonous Tumors to Radiation, Hyperthermia, and Their Combination in C3H/He Mice

Treatment	No. of mice	Survival period after treatment (mean±SD) (days)	Tumor growth time (TGT) (mean±SD) (days)	No. of mice showing		
				Complete regression	Cure	Lung metastases
Control	10	28±10	15±3	0	0	0
HT alone	6	34±10	15±6	0	0	0
20 Gy	7	40±10	25±4	0	0	0
20 Gy+HT	5	45±9	29±7	0	0	0
40 Gy	10	72±13	45±9	3	0	1
40 Gy+HT	12	65±22	47±26	5	0	1
60 Gy	14	81±28	70±29	11	0	0
60 Gy+HT	16	90±24	71±28	13	2	1

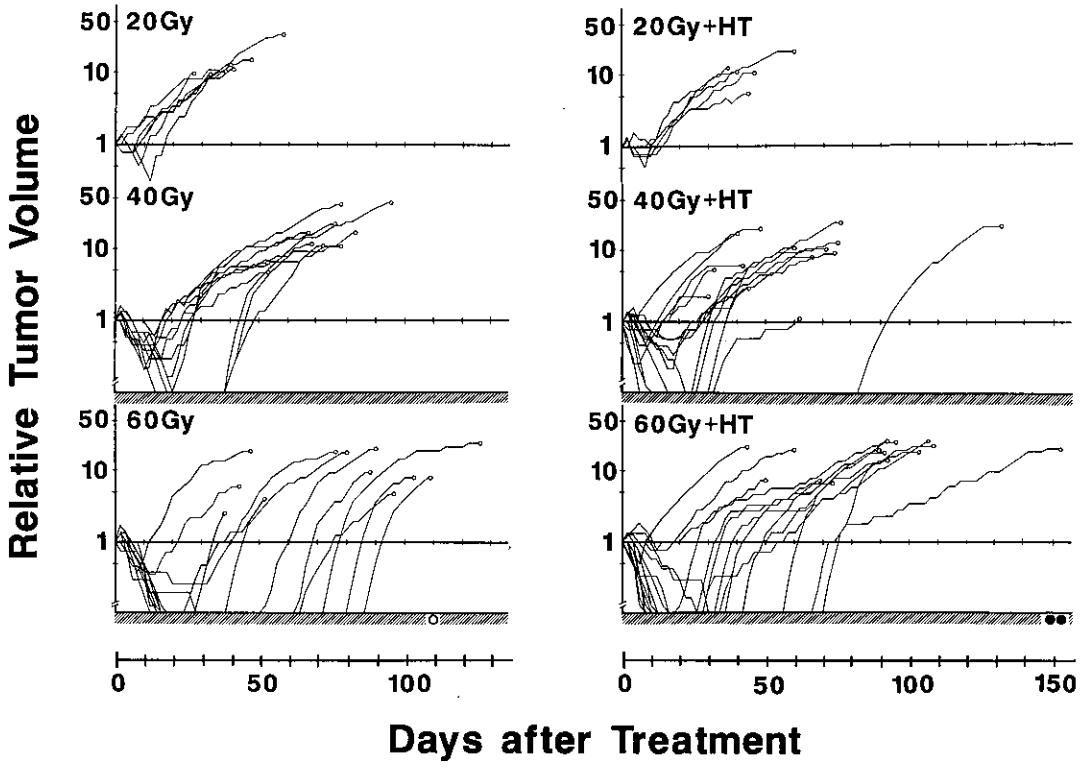

Fig. 2. Gorwth kinetics of MCA-induced autochthonous tumors in C3H/He mice, treated by 20 Gy X-irradiation, 20 Gy+hyperthermia (HT, 43.0°, 30 min), 40 Gy, 40 Gy+HT, 60 Gy, and 60 Gy+HT. Shaded parts in the graph show non-palpable tumor size. Deaths of mice are indicated by ○. Cures of mice, i.e., complete regression of autochthonous tumors for more than 120 days after treatment, are indicated by ●.

responses of autochthonous tumors to radiation, but after the combined treatment no tumor recurred within 120 days in 2 of 16 mice; i.e., they showed complete cure (Fig. 2).

Metastasis and Damage to Normal Tissue

Lung metastases were found in 3 mice with recurrent tumors (Table I). However, these metastases were not the cause of death and did not interfere with observation of the original tumors. Mild or moderate damage to normal tissue, local burns, hair loss and shortening of treated feet, were seen in groups treated with high doses of radiation. Hyperthermia enhanced the usual tissue damage caused by radiation, i.e., local burns, desquamation of skin, and loss of toes. However, this treatment did not result in severe damage such as loss of a foot.

Discussion

There are only a few reports on the effect of hyperthermia on spontaneous or autochthonous tumors in experimental animals.[5,6] Because of the technical difficulties in concentrating heat on autochthonous tumors without heating the whole body, most experiments have been carried out on transplantable tumors inoculated into the feet of mice, which can be relatively easily heated without killing the mice by overheating them. However, transplanted tumors are abnormally susceptible to various treatments and so have given over-optimistic results on the effects of therapy.

The effectiveness of hyperthermia combined with X-irradiation on autochthonous tumors was demonstrated in a randomized study on spontaneous tumors in cats and dogs by Dewhirst *et al.*[10-12] In their experiments, some spontaneous tumors showed no response to heat alone, but in combination with radiation, hyperthermia increased the response rate of tumors, and even with large tumors, some complete remissions were observed. Spontaneous tumors in pet animals are considered to be similar to human tumors with respect to tumor response to hyperthermia alone. However, with spontaneous tumors in pet animals, variations in tumor sizes, sites of origin, and histological types, and in the temperature in the heated tumor irradiated with radio-frequency waves were technically inevitable. The use of MCA-induced mouse autochthonous tumors is a feasible method for avoiding these variations.

Autochthonous tumors in mice induced by MCA are reported to be resistant to radiation,[7] chemotherapy[13,14] and immunotherapy.[15,16] Furthermore, the tumors that recurred after irradiation treatment of MCA-induced tumors were shown to be true recurrent tumors, not newly initiated tumors, by studies on marker enzymes in primary and recurrent tumors in mosaic mice.[17] In these mosaic mice tumor recurrence was observed even after treatment with a well-collimated X-ray beam generated from a 6 MeV therapeutic linear accelerator.[17] The reason for the high resistance of autochthonous tumors is unknown. But, attempts to develop a therapeutic treatment to obtain complete cure of autochthonous tumors in experimental animals are clearly very important.

In the present study, we observed many cases of complete regression of autochthonous tumors after radiation or combined treatment with hyperthermia, as has been seen with transplanted tumors. It was difficult to prevent recurrence of autochthonous tumors, i.e., to obtain complete cure, but by combined treatment with radiation and hyperthermia, we obtained a small, but significant number of complete cures. We hope to find a more efficient method for increasing the cure rate of autochthonous tumors. True cure of tumors, in a strict sense, should be achieved by this approach.

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References

1) Suit, H. D. and Shwayder, M. Hyperthermia: potential as an anti-tumor agent. *Cancer*, **34**, 122–129 (1974).

2) Hornback, N. B. "Hyperthermia and Cancer: Human Clinical Trial Experience," pp. 73–119 (1984). CRC Press, Inc., Florida.
3) Hahn, G. M. "Hyperthermia and Cancer," pp. 7–177 (1982). Plenum Press, New York and London.
4) Overgaard, J. Simultaneous and sequential hyperthermia and radiation treatment of an experimental tumor and its surrounding normal tissue *in vivo*. *Int. J. Radiat. Oncol. Biol. Phys.*, **6**, 1507–1517 (1980).
5) Marmor, J. B., Pounds, D., Hahn, N. and Hahn, G. M. Treating spontaneous tumors in dogs and cats by ultrasound-induced hyperthermia. *Int. J. Radiat. Oncol. Biol. Phys.*, **4**, 967–973 (1978).
6) Miller, R. C., Connor, W. G., Heusinkveld, R. S. and Boone, M. L. M. Prospects for hyperthermia in human cancer therapy. Part I: Hyperthermic effects in man and spontaneous animal tumors. *Radiology*, **123**, 489–495 (1977).
7) Tanooka, H., Hoshino, H., Tanaka, K. and Nagase, M. Experimental radiation therapy and apparent radioresistance of autochthonous tumors subcutaneously induced with 3-methylcholanthrene in mice. *Cancer Res.*, **40**, 2547–2551 (1980).
8) Tanooka, H., Tanaka, K. and Arimoto, H. Dose response and growth rates of subcutaneous tumors induced with 3-methylcholanthrene in mice and timing of tumor origin. *Cancer Res.*, **42**, 4740–4743 (1982).
9) O'Hara, M, D., Hetzel, F. W. and Frinak, S. Thermal distributions in a water bath heated mouse tumor. *Int. J. Radiat. Oncol. Biol. Phys.*, **11**, 817–822 (1985).
10) Dewhirst, M. W., Connor, W. G. and Sim, D. A. Preliminary results of a phase III trial of spontaneous animal tumors to heat and/or radiation: early normal tissue response and tumor volume influence on initial response. *Int. J. Radiat. Oncol. Biol. Phys.*, **8**, 1951–1961 (1982).
11) Dewhirst, M. W., Connor, W. G., Moon, T. E. and Roth, H. B. Response of spontaneous animal tumors to heat and/or radiation: preliminary results of a phase III trial. *Natl. Cancer Inst. Monogr.*, **61**, 395–397 (1982).
12) Dewhirst, M. W., Sim, D. A., Wilson, S., DeYoung, D. and Parsells, J. L. Correlation between initial and long-term responses of spontaneous pet animal tumors to heat and radiation or radiation alone. *Cancer Res.*, **43**, 5735–5741 (1983).
13) Kidera, Y. and Baba, T. Blood flow-interrupting hyperthermic chemotherapy on established autochthonous mouse sarcoma induced by 3-methylcholanthrene. *Cancer Res.*, **38**, 556–559 (1978).
14) Hosokawa, M., Mizukoshi, T., Sugawara, M. and Kobayashi, H. Therapeutic effects of PS-K and busulfan on the recurrent and metastatic diseases after the surgical removal of 3-methylcholanthrene-induced autochthonous tumors in C57BL/6 mice. *Jpn. J. Cancer Res. (Gann)*, **76**, 61–67 (1985).
15) Tokuzen, R., Okabe, M., Nakahara, W., Azuma, I. and Yamamura, Y. Suppression of autochthonous tumors by mixed implantation with *Nocardia rubra* cell-wall skeleton and related bacterial fractions. *Gann*, **69**, 19–24 (1978).
16) Suga, T., Shiio, T., Maeda, Y. Y. and Chihara, G. Antitumor activity of lentinan in murine syngeneic and autochthonous hosts and its suppressive effect on 3-methylcholanthrene-induced carcinogenesis. *Cancer Res.*, **44**, 5132–5137 (1984).
17) Tanooka, H. and Tanaka, K. Test of recurrence after experimental radiation therapy of chemically induced autochthonous tumors in mosaic mice. *Int. J. Radiat. Oncol. Biol. Phys.*, **11**, 1551–1555 (1985).