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Enhanced Tumor Cure Rates by Combination of Heat and Irradiation with a One Day Interval An Experimental Study

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Abstract—*The effects of combining heat and radiation with a* 1 day *interval were investigated. The results from more than* 500 *animals indicate that a time period between heat and irradiation may be advantageous. With heat treatment at* 42.5° C *for* 2 hr, 1 day *before irradiation, a thermal enhancement ratio of* 1.4 *for tumor cure rate was found. The* TCD_{50} *of heated and irradiated animals was* 41.1 Gy (38.6–43.7) *and the* TCD_{50} *for animals irradiated only was* 59.8 Gy (55.8–68.4).

INTRODUCTION

A GROWING number of reports indicates that a combination of hyperthermia and radiation gives a greater effect on normal and tumor tissue than irradiation alone in the same dosages [1–12]. This is of therapeutic interest only when the effects on the tumor tissue are more enhanced by heat than those on normal tissue.

Most publications show that the greatest enhancement effect is found when heat and radiation are applied with a very short time-interval between treatments [3, 4, 10, 13–15]. On the subject of extended time between heating and radiation, Overgaard found [8, 9, 16] in an *in vivo* animal tumor system no difference in enhancement with intervals up to 24 hr. There was also no influence of the sequence of the two treatments. Experiments on normal tissue damage by Myers and Field [6], with stunting of growth of baby rat tails as an endpoint, revealed that the effect of heat on radiation induced cartilage damage was only slightly enhanced when the interval between heat and X-rays was 6 hr or more. For skin damage, the results of Stewart and Denekamp [10] gave the same indications: after an interval of 3–6 hr between heat and radiation, the heat induced radiation sensitization was considerably reduced.

Okumura and Reinhold [17, 18] investigated the influence of heat on the rat skin. With heat doses at temperatures up to 43°C for 1 hr, there was no or hardly any visible damage to the skin. In combination with X-rays (20 Gy), the radiation reaction was enhanced. However, when the interval was 6 hr or more, no enhancement was found. Based on these results, a series of experiments was designed to investigate the influence of heat on the radiation effect in an experimental tumor system. A heat level of 42.5°C for 2 hr was selected because this temperature results in a minimal effect [5, 17, 18] on skin. Irradiation was performed on the following day. The assay included a total number of over 500 treated animals.

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MATERIALS AND METHODS

Materials

Male albino rats of the WAG/Rij strain 6–8 weeks of age were used. The tumor was a rhabdomyosarcoma which is isogeneous in this strain of rats. It is considered nonimmunogenic and fairly radioresistant [19]. The tumor was grown in the subcutis of the rat. Treatment was started when the tumor had reached a diameter of approximately 6 mm. The tumor was then in its exponential growth phase and had a volume doubling time of about 2 days.

Hyperthermia

For heating, the same system as described for the skin sensitivity tests [17, 18] was used. The animals were anaesthetised with hypnorm (fluanisone and phentanyl citrate, Philips Duphar) in a dose of 0.15 mg/100 g body weight. The anaesthesia was maintained with 1–2 additional doses of 0.05 mg/100 g body weight. The hair of the back was clipped and air was injected below the dorsal skin, separating the tumor bearing area of the skin from the visceral part. The result was an air pouch with a small tumor in the peripheral outside layer. The animals were placed with this dorsal air pouch in a water bath with precision controlled temperature (Fig. 1). The temperature of some tumors during the bath was measured with thermocouple needles of 0.5 mm thickness. Tumor temperature ranged from 42.0° to 42.4°C (usually 42.3°C). The water temperature was 42.5 ± 0.1°C. The air pouch prevents the body temperature of the animals from becoming too high. Rectal temperature rises slowly during the bath to about 39°C.

Irradiation

For irradiation, a 300 kV X-ray machine was used (Philips–Mueller MG 300, HVL 3 mm Cu, exposure dose rate about 380 R/min, SSD about 18 cm). The animals were irradiated one day after heating. They were anaesthesized with Nembutal (pentobarbital, 6 mg/ml, 1 ml/100 g body weight). The dorsal skin was again insufflated with air. The tumor and neighbouring skin were irradiated bilaterally. The air pouch conveniently prevented the essential normal structures, e.g., the dorsal myelum, from being irradiated. A dose range of 22–53 Gy was used for heated and 27–66 Gy for unheated tumors.

RESULTS

Skin

On the day following a heat exposure of 2 hr at 42.5°C, the skin is edematous and hyperemic. This reaction disappears in 2 or 3 days. The regrowth of the clipped hair is delayed after this heat dose [17, 18]. No further effects are obvious. When the heat treatment is given one day before the irradiation, the degree of desquamation is similar to the one observed after X-rays alone (27 Gy and higher). However, in the case of preceeding heat treatment, the skin reaction starts earlier and lasts longer. The reactions with and without heat are not completely comparable due to the different time courses. This means that an accurate 'thermal enhancement ratio' for desquamation cannot be derived. The only conclusion is that there seems to be a slightly more intense reaction of normal tissue to radiation after heat application, even after a 24-hr interval.

The skin of 'cured' animals, at 100–150 days after treatment, shows no apparent difference between heated plus irradiated and irradiated only rats. We are presently investigating the influence of this treatment on skin reactions separately, especially with regard to possible late effects.

Irradiation only

After a dose of radiation below 54 Gy, the tumor volume continues to increase for a few days. Then, the tumor stops growing and slowly regresses, sometimes becoming unde-

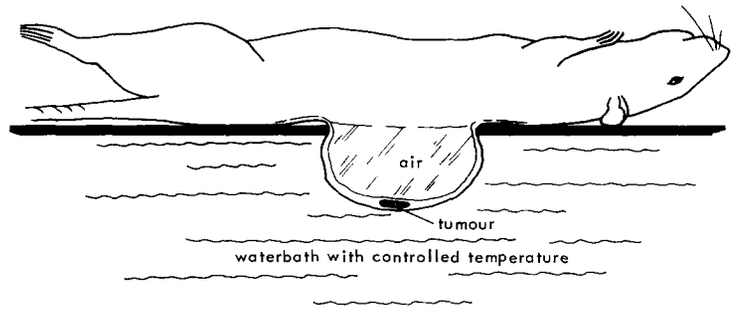

Fig. 1. Technique of heating. The dorsal skin with the tumor is immersed in the bath. The air prevents the body temperature from becoming too high. The tumor temperature is about 0.2°C lower than the water temperature.

tectable. When no cure is achieved, the tumor again increases in volume after some time, usually at a slower rate than before irradiation.

Heat only

After treatment with 42.5°C for 2 hr, the tumor stops growing immediately. There is no or only a slight decrease in volume and the tumor starts to grow again after a few days. About 6 days after treatment, the starting volume is reached and the tumor resumes growth at the same rate as without therapy (Fig. 2).

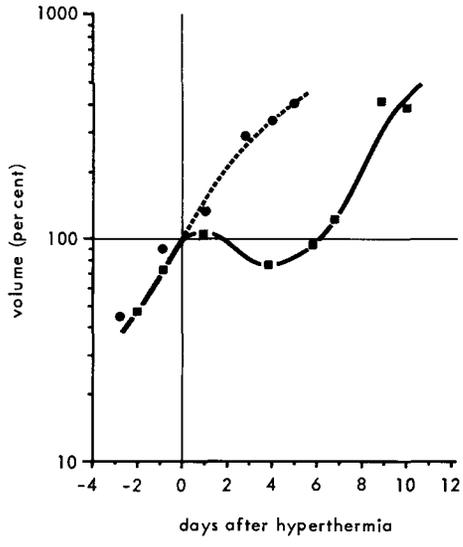

Fig. 2. Delay in tumor growth following heat treatment. Untreated tumors reach 200% *of their starting volume in about* 2 days. *Tumors treated with* 42.5°C *for* 2 hr *reach* 200% *of their starting volume in about* 8 days. (●---●) *controls;* (■——■) *heated tumors.*

Combination

The most striking effect after combination therapy is the rapid decrease in volume (Fig. 3). Most tumors become hardly detectable or not detectable at all. The rate of regression of tumor volume after combination therapy is consistently increased, as compared with that of tumors treated with radiation only. The mean starting volume is reached on a later day. There is a wide variation in the time of starting regrowth both with and without hyperthermia. The growth delay therefore cannot be calculated accurately. 'Cure' was defined as the absence of palpable growth 100 days after treatment. In earlier experiments [19], it was established that recurrence after 100 days was rare. Below doses of 35 Gy, very few animals were cured. With higher doses, significantly more animals were cured after hyperthermia and irradiation than after irradiation only.

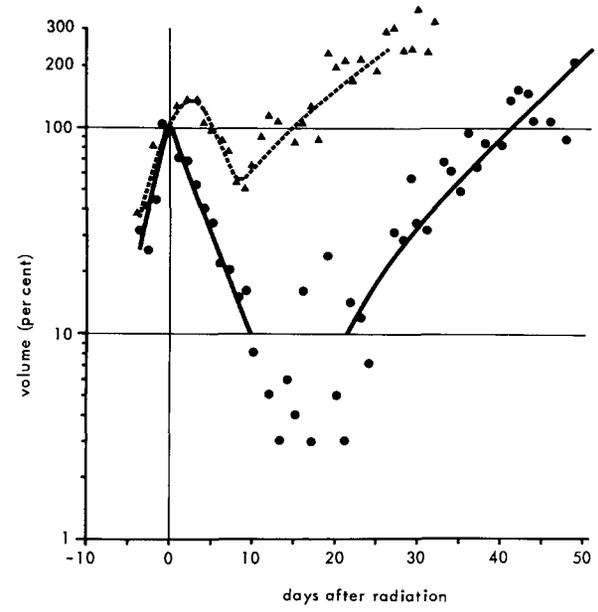

Fig. 3. Regression in tumor volume after radiation with or without heat. After a dose of 26.6 Gy, 1 day *after* 42.5° C *for* 2 hr, *there is a sharp decrease in volume. The average starting volume is again reached at* 38 days; *without heat, this occurs after* 13 days. (▲---▲) 26.6 Gy *at* day 0; (●——●) 42.5°C day −1. 26.6 Gy day 0.

The data were subjected to probit analysis. Fifty per cent cure rate without heat was found at 59.8 Gy (95% confidence limits: 55.8–68.4), with heat at 41.1 Gy (95% confidence limits: 38.6–43.7). From these data, a thermal enhancement ratio of 59.8/41.4 = 1.45 could be derived (Fig. 4).

Histological observations

Immediately after hyperthermia in the dosage used, the skin shows hyperemia, with a network of distended vessels around the tumor. The tumor has a bluish appearance due to hemorrhages. Edema develops soon after treatment and reaches its peak at one day. This reaction disappears during the next few days. When the tumor is excised and sliced shortly after heat treatment, cellular destruction can already be seen. Changes are extensive on the next day. There is interstitial edema and the cells have shrunk and show condensed nuclei and vacuolisation. Very few cells seem to be unaffected. These observations are in accord with those of Overgaard [7]. The healthy appearing cells are distributed throughout the entire tumor.

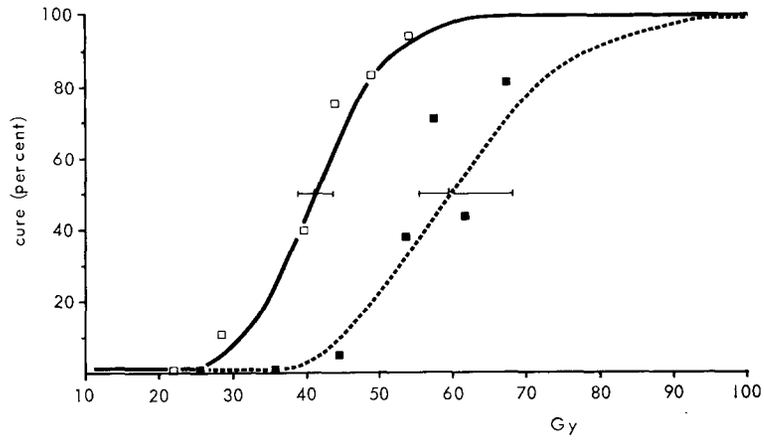

Fig. 4. Cure rates with hyperthermia plus radiation and radiation only. The percentages of cured animals for each dose are plotted. (□——□) with 42.5°C for 2 hr at day −1; (■- - -■) without heat. Calculated TCD_{50} (probit analysis) is 41.1 (38.6–43.7) Gy with hyperthermic pretreatment and 59.8 (55.8–68.4) without heat.

Proliferation starts again very soon. On the third day after heating, many histologically viable appearing cells can already be seen.

If the sequence is reversed and the tumor is first irradiated and heated one day later, there is also a gain in tumor reaction. Tumor volume regresses steeply as found in the first heating experiment. However, this treatment results in definitely more recurrences with tumors heated after irradiation than with tumors heated before. The treatment results in a TCD_{50} of 49.6 Gy (46.5–54.2). This gives a thermal enhancement ratio of only 1.2.

Lower temperature

With a heat dose of 42°C for 2 hr one day before irradiation, regression seems somewhat less steep than with the 42.5°C experiments. The cure rate is higher than with irradiation alone, as indicated by a 50% cure rate (TCD_{50}) of 48.3 Gy (95% confidence limits: 44.5–54.0), which gives a thermal enhancement ratio for cure of 1.2. At 42°C there is hardly any evidence of edema of the skin. Modification of the irradiation skin damage is not apparent.

DISCUSSION

The observations indicate that there is a definite gain in tumor reaction by adding heat to radiation with an interval of 1 day. Tumors treated by combination therapy show faster regression and the recurrence free interval is longer than of those treated by irradiation only. There is also a greater percentage of cures than with the same dosages of irradiation without heat, TCD_{50} respectively, 41.4 and 59.8 Gy. This indicates that more tumor cells are killed with combined treatment than with irradiation only.

There are at least two possible explanations for these results. When treatments are additive, cells could be killed by either modality and in this way hyperthermia killed cells will contribute to the total number of killed cells. On the other hand, heat could potentiate the killing effect of irradiation, for example, by influencing the environment of tumor cells or by interfering with repair mechanisms [2, 5, 14, 20]. Obviously, a combination of the two may also occur. As a third possibility, there can be an indirect effect of heat on tumor growth due to changes in vascularity in the tumor bed. That the first explanation is likely to be the case is shown by histologic study: a great number of cells are killed by heat alone. This does not rule out the possibility that the second phenomenon may be operative on those cells that have escaped destruction by heat. When treatments are purely additive, one would expect the enhancing effect of a fixed heat dose to become less on increasing the dose of irradiation. The present results do not point in that direction. The enhancement

ratio (cure) seems to remain more or less the same for the different dosages tested. It is conceivable that the interaction of heat and irradiation is more complicated than can be explained by just one effect.

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