Hyperthermic Treatment of Human Tumors Heterotransplanted in Nude Mice

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Abstract

A new model is presented for the study of the effects of supranormal temperatures on human tumors in vivo. Human tumors heterotransplanted serially in nude mice were heated in vivo by means of local radio-frequency heating. A lung carcinoma, a breast carcinoma, a colon carcinoma, and a malignant melanoma were studied. The tumors were transplanted s.c. in the inguinal area or under the kidney capsule of adult nude mice.

The s.c. tumors were heated for 30 min. Temperatures of 43–44°C were reached in the surrounding normal tissues, whereas in the center of the tumor temperatures of 46–49°C were recorded. In 11 of 16 randomized pairs of mice, the growth of the tumor treated by hyperthermia was inhibited by 75% or more as compared with the growth of the untreated tumor control. No mortality and only temporary damage to skin and muscle were observed.

The kidney tumors were also treated for 30 min, but it was possible to reach only 40°C in the abdomen. Seventy-five % mortality was observed. Of seven randomized pairs evaluated, five exhibited a reduction of growth varying from 37 to 63%.

The model proposed appears to be a workable and promising one, especially for s.c. growing tumors.

Introduction

A selective antitumor effect of hyperthermia applied in vivo (44–45°C) to transplantable rat tumors by means of a diathermy apparatus was demonstrated by Westermark (25) in 1927. These results were confirmed by Johnson (15), Overgaard and Okkels (18), and Crile (3) using transplantable rat and mouse tumors heated by diathermy and by immersion in thermoregulated water baths.

Studies on the antitumor effects of supranormal temperatures received a powerful stimulus by the demonstration that heat alone (2) and in association with chemotherapeutic agents (23), can induce total regressions of human tumors and increase in the long-term survival of patients so treated (24).

It was clearly demonstrated in vitro that tumor cells are more thermosensitive than are their normal counterparts even if they divide at the same rate (7). This characteristic thermosensitivity of neoplastic cells is present in both rodents (7) and in humans (9).

Numerous studies on the effects of hyperthermia applied to transplanted rodent tumors followed which are summarized by Dickson (4). There is still lacking, however, a good experimental model which would allow extensive experimentation in vivo on the effects of hyperthermia applied to human tumors.

The appearance of the nude thymusless mouse (5, 19)

which, lacking rejection phenomena, can accept heterotransplants of human tumors obtained directly from the patient (21) or grown in tissue culture (12), makes it possible to treat human tumors outside the body of the patient of origin with chemotherapy (10, 20) and radiotherapy (14).

In the present paper, we are attempting to build a model for the assessment in vivo of the thermosensitivity of human tumors serially propagated as heterotransplants in nude mice.

Materials and Methods

Nude Mice. Homozygous nude mice of both sexes bred in our laboratory and maintained under strict pathogen-free conditions (8), aged 3 months or more, were used for the present experiments.

Tumors. Human tumors representative of some of the major classes of human cancers established as heterotransplants in nude mice in our laboratory were used. These included the CO 1 lung carcinoma, a histologically poorly differentiated squamous cell carcinoma originating from a primary lung carcinoma that was removed in 1977 from a 75-year-old male. It is a fast-growing tumor in nude mice, reaching a volume of 1000 cu mm in 9 days. It has already undergone 15 passages in our laboratory.

The WILL melanoma, histologically an amelanotic malignant melanoma originating from an axillary metastasis of a cutaneous melanoma, was also used. The metastasis was removed in 1976 from a 29-year-old female. It is a slower-growing tumor in nude mice than CO 1, reaching a volume of 1000 cu mm in 15 days. It has already undergone 19 passages in our laboratory.

The third tumor was the BE 1 colon carcinoma, histologically an adenocarcinoma originating from an omental biopsy of a diffused carcinomatosis of the abdomen secondary to a carcinoma of the colon. It is a fast-growing tumor which reaches, in nude mice, a volume of 1000 cu mm in 7 days. It has already undergone 13 passages in our laboratory.

Also used was the CL 1 breast carcinoma, histologically a poorly differentiated infiltrating duct cell carcinoma originating from a primary breast carcinoma in a 29-year-old woman. It has been passaged for 33 passages in nude mice reaching a volume of 1000 cu mm in 15 days.

The said tumors were treated as s.c. transplants or as renal transplants.

To obtain suitable s.c. tumors, 0.5 ml of a fine mince of the tumor in complete tissue culture medium (Eagle's minimal essential medium; Grand Island Biological Co., Grand Island, N. Y.), 10% v/v was inoculated s.c. in the inguinal area of groups of nude mice.

Once the tumors of the group reached 500 to 1500 cu mm, the mice were divided at random into couples. One of the tumors of each couple was treated; the tumor of the other mouse was left as control.

To obtain suitable renal implants, tumors were dissected into square fragments of 2 to 2.5 sq mm in size. Nude mice were...
anesthetized with Avertine [2.5 g of tribromoethanol dissolved in 50 ml of amylene hydrate (Aldrich Chemical Co., Milwaukee, Wis.) solution diluted 1:40 with distilled water]. An i.p. injection of 0.6 ml gives 20 min of deep anesthesia in a 30 g mouse.

The abdomen was opened by a dorsal incision, the right kidney was exposed, the capsule was opened, and a single fragment of the tumor was inserted firmly beneath the renal capsule near the cranial pole of the organ. The dimensions of the inserted fragment were then again precisely measured under a dissecting stereomicroscope equipped with a micrometer ocular. The wound was sutured with surgical silk, and the animals rested for 3 days. On the day of treatment, the animals were divided at random into couples. One animal in the couple was treated, and the other was left as control. This technique is a slight modification of the one originally devised by Bogden (1). In the original technique, devised to test chemotherapeutic agents, treatment was initiated 24 hr after tumor inoculation. In our modification, devised also for testing antitumor drugs (11), treatment is initiated 3 days after tumor inoculation. This is done to allow the neoplasms to become fully vascularized, which most are by the third day after inoculation.

**Hyperthermic Treatment**

A. s.c. Tumors. The animal was anesthetized with Avertine, and a small incision was made in the back on the side of the tumor at the level of the anterior leg. A chemically sterilized alcohol thermometer was then inserted in the cut and slid s.c. until the bulb was level with the tumor mass and to the right or left of the tumor (Fig. 1). The thermometer was graduated in Fahrenheit degrees and had an accuracy of ±0.5°F or about ±0.15 to 0.2°C. This allowed us to control accurately the temperature of the normal tissues surrounding the tumor.

The tumor and the surrounding area were then sandwiched between 2 copper capacitative applicators, 3 cm in diameter, connected by cable to a radio-frequency generator operating at 13,560 MHz, crystal controlled with a power output of 50 watts (Fig. 2). To insure contact with the skin, both applicators were wrapped in gauze tissue and soaked continuously with a 0.9% NaCl solution. Standard treatment consisted of maintaining the temperature of the normal tissues surrounding the tumor at 110—112°F (43—44°C) for 30 min. Measurements of the tumor temperature were performed by reducing to 0 the output of the generator and then inserting into the tumor mass a small thermistor carried in the tip of a 22-gauge hypodermic needle connected by cable to an electric thermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio.).

B. Renal Tumors. The same basic protocol as for the s.c. tumors was used with the following modifications: (a) the thermometer was inserted under the skin of the back of the mouse with the bulb resting in the kidney area or in the rectum; (b) one applicator was placed under the abdomen and one on the back of the mouse; (c) the temperature was maintained at 104°F (40°C) for 30 min.

**Assessment of Results.** Tumor volumes s.c. were measured twice a week in both treated and control animals. Selected animals were sacrificed and their tumors examined histologically.

Renal tumor-carrying animals were sacrificed 11 days after tumor inoculation, and the tumor area was measured under the same stereomicroscope. The kidney containing the tumor was then fixed in formalin and examined histologically.

### Results

The quantitative results obtained by hyperthermic treatment of s.c. tumors are summarized in Table 1. Examples of the growth curves of representative randomized couples are given in Chart 1 for melanoma WI 1 and in Chart 2 for lung carcinoma CO 1.

The results obtained show a considerable degree of variability. However, in 11 of 16 randomized pairs, the tumor treated by hyperthermia had its growth inhibited by 75% or more as compared with the growth of the untreated tumor control. In 5 of the treated mice the tumors barely grew at all, and in 4 the tumor mass actually regressed. Temperatures inside the tumor mass varied, but in every case were higher than in the neighboring normal tissues by 3—5°C (recorded temperatures 46.7 to 49.5°C inside the tumor, and 43.3 to 44.4°C in the neighboring normal tissues). No significant differences were observed among the 3 human tumors studied. No treated animal died during hyperthermia or during the following 14 days. The skin covering the tumor mass and the area immediately adjacent was damaged by the treatment but always recovered. In some cases, a temporary impairment in the movement of the leg adjacent to the treated tumor was observed. In every case, the leg was again moving normally within 2 to 3 days.

Fig. 3 shows the histology of an untreated control lung carcinoma (CO 1). Fig. 4 shows the histology of a CO 1 tumor treated by hyperthermia 12 hr before fixation, and Fig. 5 shows the histology of a CO 1 tumor treated with hyperthermia 11 days before fixation, which regressed completely.

Histopathological examination (Fig. 3) of the tumor implant before treatment revealed an anaplastic squamous cell carcinoma. The neoplastic cells had rounded vesicular nuclei with frequent mitoses. The tumor cells showed grouping with epithelial cohesion. Adjacent to the area of cohesion were tumor cells without cohesion with a sarcomatoid appearance. Examination (Fig. 4) of the tumor implant 12 hr after treatment showed no evidence of degeneration. The histological features of the neoplastic cells were identical to the untreated tumor implant. Frequent mitoses were still present. Examination of the tumor implant site (Fig. 5) 11 days after treatment revealed no residual tumor cells. Sections of the implant site demonstrated loose fibroblastic tissue infiltrated by inflammatory cells.

The quantitative results obtained by hyperthermic treatment of human tumors are summarized in Table 2. These results show some variability. However, in 5 of 7 randomized pairs, the tumor treated by hyperthermia had its growth inhibited by 37 to 63% as compared with the growth of the untreated tumor control.

It was impossible to raise the temperature above 40°C in the abdomen without killing the animal. Even at 40°C, a 30-min treatment caused a high mortality. Of 28 animals so treated, only 7 survived until sacrifice 8 days after hyperthermia.

### Discussion

The induction of supranormal temperatures in the limbs of cancer patients has proved a valuable form of antitumor therapy, at least in cases where the majority of the neoplastic cells
Effect of hyperthermic treatment on the growth of human tumors heterotransplanted s.c. in nude mice

Table 1

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<th>Tumor</th>
<th>Treatment</th>
<th>Duration (min)</th>
<th>Temperature (°C)</th>
<th>V₀ (cu mm)</th>
<th>V₁/V₀* (7 days)</th>
<th>V₁/V₀* (14 days)</th>
<th>% tumor inhibition at 14 days</th>
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b Days after treatment.

% inhibition = 100 - \( \left( \frac{V₁/V₀ (treated)}{V₁/V₀ (control)} \times 100 \right) \)

were still within the treated area (2, 23, 24).

The best results have been obtained with local heating by closed-circuit hyperthermic perfusion of the affected limb coupled with local chemotherapy (23, 24). With this type of treatment, high temperatures can be safely maintained with minimum fluctuations in the treated area. The antitumor activities of hyperthermia and the chemotherapeutic agent used (phenylalanine mustard-Alkeran) are at least additive (6).
All of these studies have as their basis the demonstration at the cellular level that tumor cells are more sensitive to supra-normal temperatures than normal cells (2, 7, 9).

A further impetus to research on the antitumor effect of hyperthermia has been given by the finding of LeVeau et al. (16) that human malignant tumors are preferentially heated if the area of the body in which they grow is placed in an electromagnetic field of 13.5 MHz frequency and of sufficient intensity. Actually, the tumor is not heated any more than the surrounding normal tissues, but it is cooled less by the blood circulation. Malignant tumors (human and animal) have a much reduced blood flow per unit of volume as compared to normal tissues and organs (13, 16).

To study experimentally the effect of radio-frequency heating, an in vivo system is needed because this differential heating of the tumor tissue presupposes the existence of blood circulation. The possibility of using human tumor heterotransplants for the study of antitumor therapies is being actively investigated at present (10, 14, 17, 20, 22). We thought that the same system with small modifications could be used to study the effect of hyperthermia on the human tumors alone and in combination with other forms of treatment.

The results obtained, although preliminary, have already shown that even single applications of hyperthermia for the short period of 30 min can have a profound effect on the growth of human tumors in this system. The preferential heating of the tumors by radio frequency has been confirmed (3 to 5°C higher temperatures have been observed in the tumor as compared with the surrounding normal tissues).

A large variability in thermosensitivity has been observed between limbs and abdomen. The first can easily tolerate 43—44°C, the second barely 40°C (with a high consequent mortality).

Interestingly, at the temperature achieved, the antitumor effect obtained does not seem to vary much from tumor to tumor.

In conclusion, it seems that the model devised to study experimentally the effect on human tumors of radio-frequency heating has proved to be a workable one. It remains now to establish if the results obtained in such a model can be quantitatively transferred to tumors in the human patient. If this is proved, then such a model can provide valuable information on the optimal temperature and length of treatment necessary to achieve eradication of human tumors.

References
11. Giovanella, B. C., Stehlin, J. S., and Shepard, R. C. Experimental chemotherapy of human malignant tumors transplanted subcutaneously and under...


Fig. 4. Section of tumor CO 1 twelve hr after hyperthermic treatment. The neoplastic cells appear to be identical to the cells of the untreated tumor. H & E, × 400.

Fig. 5. Section of tumor CO 1 eleven days after hyperthermic treatment. No residual neoplastic cells are visible. The site is now composed of loose fibroblastic tissue infiltrated by inflammatory cells. H & E, × 250.
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