Tumor Cure and Cell Survival after Localized Radiofrequency Heating

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SUMMARY

Radiofrequency electromagnetic fields at 13.56 MHz were used to heat locally EMT-6 sarcomas and KHJJ carcinomas in BALB/cKa mice. Temperature profiles obtained in tumors during treatment showed uniform temperature distribution throughout the tumor volume with no systemic hyperthermia. Temperature could be maintained at a stable level throughout treatment by adjustment of power. Tumors were treated at 43°, 43.5°, and 44°, for 5, 10, 20, 30, and 40 min. The EMT-6 tumor was highly sensitive to cure by radiofrequency heating: a 5-min exposure at 44° resulted in cure of almost 50% of the tumors. Cure rate was a function of temperature and of duration of exposure. The KHJJ carcinoma was somewhat more resistant to cure by radiofrequency heating, although most of the animals treated at 43.5° or above were cured of their tumors. In an effort to explain the remarkable effectiveness of radiofrequency heating, tumor cell survival studies were done on EMT-6 tumors treated in situ. Cell inactivation by radiofrequency heating was similar to that for hot water bath heating. However, direct cell killing cannot account for the observed cures, and an additional mechanism must be responsible for tumor eradication.

INTRODUCTION

Clinical observations that heat may be an effective modality in causing tumor regression go back many years (14). One of the difficulties in exploring the role of heat in cancer therapy has been a lack of adequate methods to heat tumor volumes accurately and precisely. Methods of local heating include hot water baths (3), short-wave diathermy (11), RF-induced currents (5, 9), microwaves (1, 10), and ultrasound (2, 7, 8, 15). These previous studies have shown varying success in treatment of animal tumor models. In this study, we have utilized RF-induced currents to locally heat flank tumors in mice. Intratumor and systemic temperature were followed in all animals, and temperature distribution within the tumor and adjacent normal tissue was mapped. The 2 tumors used were highly sensitive to cure by RF field heating. We therefore initiated experiments in which tumor cell survivals were assayed in vitro after in vivo RF treatment of the tumors. The cure data could not be correlated with the cell inactivation results, indicating that host response must be, in part, responsible for tumor eradication.

MATERIALS AND METHODS

Heating Equipment. The equipment for heating of flank tumors by RF fields was supplied on a proprietary basis by Critical Systems, Inc., Palo Alto, Calif. (patent applied for). The block diagram (Chart 1) depicts the circuitry of this instrument. It consisted of a portable RF signal generator with an unmodulated output of 13.56 MHz with coarse and fine adjustments to control power output. Meters were used to monitor voltage and current of the output signal. Data on voltage and current were used to calculate the "impedance" of the mouse during treatment. Two insulated metal electrodes acted as capacitor plates between which the alternating field was impressed. The lower electrode was 10 cm in diameter; the upper electrode was 1.2 cm in diameter. Capacitors were present in the handles of the electrode "paddles," and small inductive coils tuned the circuit. This was done to maximize the resistive (and hence heating) component of the current generated in the tissue. The mouse was placed on the 10-cm electrode with the tumor up, and the 1.2-cm electrode was coupled directly to the skin above the tumor (see Fig. 1). With this electrode configuration, currents flowing from one electrode to the other had highest density in the tumor volume and adjacent normal tissue. Hence, maximum heating occurred in this region. The current density was reduced progressively away from the tumor as the large electrode was approached, resulting in less deposition of energy per unit volume and, hence, less temperature rise.

Temperature Measurements. Commercially available thermistors embedded in the tips of 24-gauge needles were used as temperature sensors (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). The leads were shielded in order to minimize RF pickup. The use of conducting needles entailed some technical difficulties: the presence of the conductor tended to concentrate the current density in the neighborhood of the metal, thus raising the local temperature and giving false impressions about the tissue temperature away from the sensor; in addition, eddy currents...
inoculation of 10⁷ viable tumor cells and were used for treatment approximately 10 to 12 days later when tumor size reached ~100 cu mm (8 to 10 mm, longest diameter). At the time of treatment, animals were anesthetized with Diabutal (50 to 60 mg/kg). The tumor and opposite flank were shaved and conducting electrode cream was applied. The mouse was secured on the 10-cm electrode with the tumor up and a 24-gauge thermistor probe was inserted into the tumor at right angles to the electrical field and as near to the center of the tumor as possible. The upper (1.2-cm) electrode plate was then centered over the tumor and contact was made with the skin above the entire tumor. Temperature was monitored throughout the treatment period. Power output was adjusted to keep temperature at the desired level. Voltage and current readings and impedance data were kept on each animal. Animals were checked for tumor size, regrowth, burning, and change in body weight twice a week. In 1 series of experiments, animals were sacrificed at varying times after treatment and tumors and normal tissues were studied histologically. "Cure" was defined as complete disappearance of all visible and palpable tumor and regrowth of normal hair over the tumor site for a period of longer than 30 days. No recurrences were noted later than 30 days, although many animals were followed for more than 120 days. All untreated tumor-bearing animals died with massive tumor growth.

In vitro Cell Survival Assay. In these studies, tumor-bearing animals were treated by RF fields as described above. For those studies in which water bath heating was used, tumors were implanted in the thighs of animals and the entire leg was immersed in a water bath 0.5° higher than the desired tumor temperature. Tumor core temperature was monitored throughout. Identically treated tumors were excised immediately, pooled, and minced, and a single cell suspension was made. Survival of tumor cells was assayed by cloning as previously described (12, 13). Control tumors were from animals treated identically but without application of heat. Surviving fraction was defined as cloning efficiency of treated tumors divided by the cloning efficiency of control tumors.

RESULTS

Temperature Mapping. In several animals, temperature was recorded at multiple sites in the tumor, adjacent normal tissue, and rectum during RF treatment. A typical temperature-time profile for an EMT-6 tumor is shown in Chart 2. Rectal and tumor temperatures were initially low due to the Diabutal anesthesia but rose immediately when power was applied (Chart 2, hatched lines). Tumor temperature rose much more rapidly than body temperature, and 43° was reached within 5 min. Temperature was maintained by adjustment of power. Typical initial incident power levels were 0.9 watts/sq cm. Power was then adjusted downward to maintain the tumor temperature at 43.5°. Average maintenance powers were of the order of 0.6 watts/sq cm. Host body temperature rose slowly during treatment but, in the case shown, did not exceed 36°. Body temperatures exceeding 37° were never observed.

Temperature measurement at various depths in the tumor...
and in normal tissue confirmed that localization of tumor heating was due to the geometry of the electrodes and location of the tumor. Probes placed at different points in the tumor where current density was high and symmetrical showed uniform heating. Probes inserted into muscle tissue adjacent to the tumors usually registered temperatures about 0.5° lower than tumor temperatures, although occasionally muscle temperature was equal to, or even higher than, that of the tumor. Temperatures in tissue adjacent to the large electrode where current density was low were the same as rectal temperatures.

Decay of intratumor temperature after the power was turned off extrapolated back to the thermistor readings obtained during RF treatment, indicating little interference with thermistor readings by the RF field.

Host Effects. Treatment for 30 min or more at 44° resulted in loss of 1 to 2 g of body weight in the 4 days following treatment (5 to 10% of body weight). Treatment at lower temperatures, or at 44° for shorter times, did not result in significant weight loss. The death rate associated with treatment and anesthesia for exposures of 20 min or more was 3% at 44°, 3% at 43.5°, and 2% at 43°, based on treatment of more than 100 animals at each temperature. At autopsy, some of these animals showed erythema and edema of the intestine underlying the tumor. No animals exposed for 10 min or less died.

Microscopic examination of tissue from a series of animals sacrificed after treatment at 44° showed small areas of focal necrosis in liver, kidney, or gut directly underlying the tumor in about 5% of the animals treated. The remainder appeared microscopically normal. These studies are being continued in more detail with examination of both normal tissue and treated tumor.

Tumor Cure Experiments. The EMT-6 mammary sarcoma and the KHJJ carcinoma differ both in immunogenicity and in sensitivity to cure by X-irradiation. The TCD_{50} for the KHJJ carcinoma is about 5350 rads, and it has no identifiable immunogenic features; the TCD_{50} for the EMT-6 tumor is about 2900 rads and it is slightly immunogenic (12, 13). RF heating was extremely effective in curing both these tumors. Chart 3 shows the cure rate at 3 different temperatures for various exposure times for EMT-6 sarcomas. All animals were cured at 44° by exposures of 20 min or longer. Approximate 50% cure rates were achieved with 5 min of exposure at 44°, with 10 min of exposure at 43.5°, and with 30 min of exposure at 43°. Thus, at lower temperatures, longer exposure periods are required for similar effects.

Tumors that received "adequate" heating (43.5°-44° for >20 min) and failed to be cured usually grew back from a single edge as if one area of tumor had not been heated adequately. Thus, it appears that it is the lowest tumor temperature which determines the ability of the tumor to regrow. Cells from tumors that grew back late after treatment were tested for heat resistance in vitro. Their heat response was similar to that of controls, suggesting that a heat-resistant subpopulation was not responsible for treatment failure.

The KHJJ tumor was more resistant to cure than the EMT-6, although a significant proportion of cures could be obtained with adequate heating (Chart 4). At 43.5° and 44°, 20-min exposures were required to produce a 50% cure rate, and 100% cure rates were not achieved.

Tumor Cell Survival. Tumor cell survival in treated tumors was assayed in an effort to explain the unexpected sensitivities of these tumors to cure by RF-induced hyperthermia. In the 1st series of experiments, cell survival as a function of temperature was evaluated in RF-heated EMT-6 tumors. Temperatures ranged from 41.5-44°. Following treatment,

tumors were excised immediately and the surviving fraction was assayed. Controls that had been sham heated also had their tumors excised, and cells from these tumors were used as plating efficiency controls. The results of these studies are shown in Chart 5. Significant tumor cell kill was achieved at temperatures well tolerated by the host. At 42° and above, the cell survival fell significantly at each successively higher temperature. At 44°, survival of clonogenic cells in the tumor was about 4%. These results were similar to results obtained with EMT-6 cells heated in vitro (Schneider and G. M. Hahn, unpublished data).

Chart 6 shows the effect of 43.5° hyperthermia if the duration of exposure is varied. EMT-6 tumors were heated by the RF field to 43.5° for 10, 20, 30, or 40 min. Longer exposures resulted in lower tumor cell survival. A 40-min exposure yielded a cell survival level of about 10%. Similar survival studies for EMT-6 tumors heated with water bath are shown on the same chart. There is no statistical difference between the 2 results.

In all these studies, the total number of cells obtained by trypsinization of tumors was determined; there were no systematic differences between cell yields from heated tumors and from controls. Thus, it is unlikely that cell lysis biased the results in a major way. However, because of variations in cell yield from group to group even within the controls, the possibility that preferential lysis of some heat-killed cells occurred cannot be ruled out. If the entire nonproliferating compartment (which presumably includes the nutritionally deprived, trypsin-sensitive cells) had lysed, this would reduce the surviving fraction by 50%.

DISCUSSION

Three major features emerge from the data presented: (a) RF fields can be designed to uniformly heat superficial tumor volumes; (b) RF heating is remarkably effective in curing both EMT-6 and KHJJ tumors; (c) direct cell killing by the RF heating alone cannot explain the dramatic tumor cure results.

The mapping studies showed that heating was relatively uniform throughout the tumors. It has been proposed that, owing to poor vasculature, tumors might lose heat less efficiently than normal tissue and, thus, would stabilize at temperatures above those of surrounding normal tissue (1,
Tumor Cures by Radiofrequency Heating

6, 9). While we did find an indication of this in some tumors, our results indicate that localized heating of the tumors is due mainly to the geometry of the RF field. When tumor and muscle were exposed to the same RF intensity, muscle showed temperatures only 0.5–1° less than tumor temperature at the same depth; muscle immediately adjacent to the tumor was often the same temperature and sometimes even higher than the tumor.

Tumor cures by various electromagnetic treatment techniques have been reported sporadically at least as far back as the 1930’s. The older literature on this topic has been reviewed thoroughly by Overgaard and Overgaard (11). In the older studies, temperature measurements were often omitted or were technically inadequate. Cater et al. (1), in 1966, were among the first to adequately monitor temperature, and this group noted no effect of microwave heating at 47° on a transplantable rat hepatoma. However, their exposure times were only 5 to 10 min. In careful studies, Overgaard and Overgaard utilized a modified diathermy unit to heat HB mammary carcinomas in C3H mice. They showed that cure rate was related to length and temperature of exposure. Similar cure rates were achieved, for instance, with a 120-min exposure at 42° and a 45-min exposure at 43.5° (11). More recently, Mendecki et al. (10) reported on treatment of a mammary adenocarcinoma in C3H mice with a 2450-MHz microwave applicator. They report 100% cures in 54 animals treated 4 times for 45 min at 43° tumor surface temperature (43.5° intratumor temperature).

Cytotoxic effects of electromagnetic fields (other than thermal) have been suggested in the past but have been discounted by others (4). Our cell survival studies are in agreement with the concept that cell killing by RF fields is by thermal mechanisms alone. Equivalent levels of cell killing were achieved at comparable temperatures whether the heating was by RF fields or by water bath.

Both Overgaard and Mendecki ascribed their tumor cures to hyperthermic cell killing by the electromagnetic field. Our cell survival studies indicate that other mechanisms in addition to thermal cell inactivation must be involved to explain the remarkable tumor cure results. There is relatively little killing of EMT-6 cells either in vivo or in vitro if these are heated by RF or by hot water baths. Charts 5 and 6 show that, shortly after a curative RF treatment, cell survival is 10 to 80%. For comparison, after a dose of 3000 rads of X-irradiation (approximately the TCD50), less than 0.1% of the cells survive (12). Studies with cyclophosphamide and nitrosoureas yield similar results; until cell survival is reduced to about 0.1%, few, if any, tumor cures are observed (G. M. Hahn, unpublished data). If all the cells in the nonproliferating compartment were lysed by trypsin, the maximum error in estimation of survival values in these studies would be an overestimation by a factor of 2 (see "Results"). Even this error would not put the survival in the range where radiation or chemotherapy cures are obtained. Thus, direct cell killing by the RF field cannot account for the tumor cures.

The level of cell killing seen in the tumors in vivo is very similar to that seen in vitro in plateau phase cultures at pH 7.4 (Schneider and G. M. Hahn, unpublished data). Thus, the suggestion of Overgaard and Overgaard (11) that cells in the tumor are more sensitive to heat owing to lower tumor pH or the release of hydrolases does not seem to be correct, at least for EMT-6 tumors. Delayed mechanisms, either intratumor or involving host response, must play a major part in tumor eradication. Possible mechanisms for delayed killing include selective destruction by hyperthermia of tumor blood vessels and stimulation of a tumor-directed immune response. Since the KHIJ tumor is far less immunogenic than the EMT-6 (13), the higher thermal resistance of this tumor is consistent with the concept that immune recognition after heating has a role in tumor eradication.

Understanding of tumoricidal mechanisms and of heat resistance is important for optimal clinical application of data such as those presented here. Le Veen et al. (9) have used an RF heating device almost identical to ours to heat tumors in patients. Although their study appears to have been poorly evaluated and is anecdotal in nature, a few of the cases do seem to have benefited from treatment. However, it should be pointed out that Le Veen et al. estimated that they heated tumors to 50°C, although they did not state how the tumor temperature estimates were obtained. If their estimates were reliable, then they used temperatures much higher than any in our study. In any case, RF heating may prove to be useful in the treatment of some cancers even before we understand in detail the biology of thermal cell and tumor inactivation.

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