Capacitive Radiofrequency Hyperthermia in the Treatment of Cutaneous Murine Melanoma

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We have evaluated localized capacitive radiofrequency hyperthermia in the treatment of murine S91 melanoma. Two hundred and ten DBA/2J male mice were implanted with $1 \times 10^6$ S91 murine melanoma cells inoculated into a noninflammatory upper dermal suction blister cavity. Two tumors were implanted per animal, so that each animal served as its own control in evaluating the effects of temperature, treatment duration, and tumor size on tumor growth following radiofrequency hyperthermia treatment. The data supported the following conclusions: (1) capacitive radiofrequency hyperthermia is effective in the treatment of murine S91 melanoma; (2) duration of treatment between 10 and 60 seconds at 52°C does not influence effectiveness; and (3) treatment temperatures $>49°C$ are needed for maximal effectiveness in the treatment of these tumors. Based on these preliminary findings, high temperature, short duration capacitive radiofrequency hyperthermia may prove to be a useful modality in the treatment of certain cutaneous malignancies. J Invest Dermatol 89:518–522, 1987

Hyperthermia (high temperature heating) as a cancer treatment modality is based on the premise that malignant cells are sensitive to thermal effects [1]. This premise is supported by a large body of data. Heating to temperatures above 42°C produces an increase in blood flow in normal tissues with a decrease in tumor blood flow, thus causing inefficient tumor heat dissipation [2,3]. This sluggish tumor blood flow leads to vascular occlusion and tumor necrosis [4]. Decreased blood flow following heating also results in a decrease in pH, which enhances thermal killing and inhibits the repair of heat damaged cells [5]. Inhibition of thermal repair prevents the development of cellular tolerance [6,7]. This prevents the expansion of a heat-resistant cell clone. Hyperthermia also causes cell death in those cells, thus creating a low pH environment [8,9]. This contrasts with radiation, which can only kill well oxygenated cells in a normal pH environment, making hyperthermia a valuable treatment modality in rapidly growing tumors such as melanoma where a substantial cell population has a low pH due to hypoxia [9]. In addition, hyperthermia sensitivity of cells is greatest during the S-phase of the cell cycle, the period when cells are most resistant to radiation damage. Thus hyperthermia may offer an additional tumor kill advantage when combined with radiation [10]. Other preferential tumor metabolic alterations following high temperature heating include a marked depression of oxidative metabolism, increased formation of lysosomes accompanied by lysosomal rupture, and decreased synthesis of RNA, DNA, and proteins [11]. Evidence also exists that hyperthermia causes stimulation of T cells and macrophages, which may aid in tumor regression [12,13].

Modalities to produce hyperthermia include water bath, ultrasound, radiofrequency, and microwave. The manner in which heat is produced determines the degree, depth, and pattern of heating. Water bath tumor immersion cannot produce localized heating [14]. Ultrasound (0.2-3 MHz) produces a nonuniform heating pattern [15]. Microwave (915-2450 MHz) produces extensive depth penetration [16,17]. We chose to evaluate capacitive radiofrequency (0.5-13.56 MHz) hyperthermia in the treatment of cutaneous murine melanoma since a uniform heating pattern is produced with a depth penetration sufficient to heat a dermally implanted tumor.

MATERIALS AND METHODS

Animal Model Two hundred and ten DBA/2J male mice (Jackson Labs, Bar Habor, Maine) weighing between 20 and 25 g were housed in mesh cages, 5 animals per cage, with a constant supply of standard mouse chow and water.

A subclone of Cloudman S91 murine melanoma cells has been maintained continuously for the past 5 years by growth in syngeneic DBA/2J mice and subsequent cultivation of tumor cells in 75-cm plastic flasks with 25 ml of nutrient mixture F-10, heat inactivated 2% fetal calf serum, 10% horse serum, 10 mg/ml of streptomycin, and 100 U/ml of penicillin G. The media were replaced every 3-4 days and subcultures were carried out weekly using 0.2% edetic acid [18]. Cells for in vivo implantation were always taken from one of the first three in vitro subculture passages.

The animals were intracutaneously implanted with $1 \times 10^6$ murine S91 melanoma cells. Intracutaneous implantation was accomplished by inoculating cells into an upper dermal blister cavity [18] created by a suction blister device that consisted of a 200 mmHg vacuum pump attached to a specially designed suction head consisting of five 5-mm round orifices, which were placed in contact with the shaved animal’s back. Five minutes of suction application resulted in the formation of a noninflammatory bulla in the upper dermis into which the S91 murine melanoma cells were implanted, thus mimicking a melanoma metastasis. Two tumors were implanted per animal to allow each mouse to act as its own control. Tumors were allowed to grow for time periods of 1, 3, 5, 7, 9, and 11 days after implantation before treatment.

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to evaluate the effectiveness of radiofrequency heating on varying tumor sizes, since the tumors enlarged rapidly after implantation.

The therapeutic effectiveness was assessed after 24 days of post-treatment observation. The tumor implantation sites were excised and the tumors were desiccated overnight in a drying oven and then weighed. The difference between control and treated tumor weights was used as an endpoint for evaluation of treatment response. Animals could only be followed for 24 days, since the control tumors often began to enlarge to the point of interfering with the animal’s normal functioning.

Hyperthermia Apparatus A hand-held, 2 mHz capacitive radiofrequency generator (Fig 1) (RDM Engineering, Phoenix, Arizona) was used to administer the hyperthermia. The unit was powered by a wall rechargeable battery contained within the device, which powered a 2 mHz radiofrequency sine wave amplitude modulated oscillator with a maximum 10 watt output, which sent the high frequency electric current to a bipolar surface probe. The probe was placed in contact with skin on either side of the tumor and the resistance of the tissue to current flow produced internal heat generation. The sensing arm of the probe contained a thermistor that monitored the skin surface temperature, and electronic feedback was used to control the applied power. The electrostatic field was achieved by placing the two arms of the probe on opposing sides of the tumor; thus the tissue became part of the dielectric of a capacitor. The probe arms contained compensating inductive coils tuned to the circuit to neutralize the capacitive reactance between the electrodes and the tissue to maximize the heating component of the current in the tissue. The distance between the probes was 3 mm resulting in a 3 x 3 mm square heating area. Three overlapping fields were used in our treatments resulting in a 9 x 9 mm square treatment area.

The radiofrequency unit had an initial heating period whereupon another oscillator signaled the attainment of the treatment temperature. We examined mean treatment temperatures of 42°C, 49°C, 50°C, and 52°C and 10, 30, and 60 seconds of heating. The various temperatures were obtained by increasing the power of the electric current delivered to the tissue.

Coupling between the cutaneous probe and the shaved animal’s back was accomplished by moistening the skin with normal saline, which aided in heat transfer. Depending upon the hydration characteristics of the skin containing the tumor and the content of the underlying tissue (i.e., bone or fat), the resistance of the skin can vary between 50 Ohms and 500 Ohms. Moistening the tissue decreases the resistance and provides for even surface heating. The layer of fat found beneath the dermis in skin also insures surface heating since it is thermally and electrically less conductive than moistened skin, and thus insulates the deeper tissues.

Temperature Measurement Cutaneous temperature measurements were taken by a thermographic camera and implanted thermocouple temperature sensors. The thermographic camera (Fig 2) was used to obtain mean surface temperatures over the treatment period, since both convective and conductive thermal losses caused some temperature variation. When treatment temperatures are discussed, we are expressing the mean tumor surface temperature as observed by the thermographic camera during the treatment period.

Cutaneous depth temperature was evaluated by using insulated and shielded copper-constantan miniature thermocouples and a digital readout meter (Sensortek, Saddle Brook, New Jersey) inserted through a 16-gauge catheter into a melanoma tumor. As expected, temperatures decreased as depth increased. For example, when the hyperthermia unit was set for a treatment temperature of 50°C, the surface temperature of 50°C was maintained to a depth of 3 mm with hyperthermic heating above 43°C occurring to a depth of 5 mm. A temperature of 36°C could be detected down to 13 mm. The baseline body temperature of the animal was 32°C. The superficial nature of the heating insured that full thickness necrosis of the skin would not occur.

RESULTS

Posttreatment Appearance All animals treated developed mild second degree burns at the sites of the hyperthermia treatments. Punch biopsies performed at 24 h posttreatment revealed a necrotic epidermis with viable basal cells in the hair follicles in the dermis. By 72 h posttreatment, hyperplastic epithelium was seen arising from regional follicular epithelium (data not shown). All animals healed with minimal scarring and no hyperthermia associated fatalities occurred during or immediately after treatment.

Clinical Effects of Hyperthermia

Treatment Temperature Variation: We chose to evaluate the effect of temperature on tumor growth by examining treatment temperatures of 42°C, 49°C, 50°C, and 52°C on one day old tumors treated for 30 seconds. Figure 2 demonstrates that treatment temperature affects melanoma recurrence rate. There were five recurrences in 37 animals at temperatures >49°C compared with...
eight recurrences in 15 animals at 42°C (p = 0.018). However, the mortality associated with increased tumor treatment temperatures was notable with four deaths at 52°C, two deaths at both 50°C and 49°C, and no deaths at 42°C. The deaths in these animals occurred at least 2 weeks after treatment and were not due to tumor burden since in all cases the control tumor had not necrosed and no gross evidence of tumor remained at the treatment site. The deaths could possibly be attributed to the fact that a 9 × 9 mm burn site represents approximately 5% of the animal's total body surface area, which represented an insult.

Temperatures >49°C appear to be the most effective with no significant difference between the recurrence rates for 49°C, 50°C, and 52°C. A 9% recurrence rate (1/11 animals) was noted at 52°C compared with a 23% recurrence rate (3/13 animals) at 50°C and an 8% recurrence rate (1/13 animals) at 49°C (Fig 3). Over half of the animals (53%) developed recurrences when treated at 42°C. Clearly, short duration 42°C hyperthermia is not effective in the treatment of small murine melanoma tumors. All surviving animals healed with the minimal scarring and complete hair growth over the treatment site. It is concluded that temperatures >49°C can be used successfully for short durations in melanoma treatment with rapid healing of the treated site.

A group of 20 animals were treated at 52°C for 30 seconds and followed for 113 days postimplantation. The tumors were seven days old and approximately 5 mm in diameter when treated. These animals were followed to determine the long-term response to a single hyperthermia treatment. There were two recurrences out of the 20 animals with death occurring in the animals exhibiting recurrences prior to day 113. An additional two animals without tumor recurrence at their time of death died of unknown causes. Therefore, 16 out of 20 (80%) of the animals experienced a long-term tumor cure.

**Treatment Time Variation:** We chose to evaluate the length of hyperthermia treatment in relation to tumor growth by treating one-day-old tumors at 52°C for 10, 30, and 60 seconds. The data in Fig 4 demonstrate that there is no difference in effectiveness of hyperthermia treatment between 10, 30, and 60 second treatment durations. The effectiveness of a 52°C treatment temperature again was associated with some mortality (four deaths in 15 animals). Surprisingly, no animals died at the longest 60-second treatment period, but three animals were lost at 30 second and 1 animal at 10 second treatment periods. Only one recurrence was noted in all 3 groups and this occurred with a 10 second treatment period. These data suggests that it is the initial exposure to high temperatures that causes the majority of the small tumor cell kill perhaps due to a vascular response. Shorter treatment times did offer the advantage of more rapid treatment site healing with less cutaneous scarring (data not shown). We are currently investigating these concepts in larger tumors.

We evaluated the effectiveness of radiofrequency hyperthermia on tumor size as measured in days postimplantation (tumor size). Tumors of 1, 3, 5, 7, 9, and 11 days duration were treated for 30 seconds at 52°C. As shown in Fig 5, there is a lower rate of melanoma recurrence for tumors treated in the early growth phase prior to nodule formation under 7 mm in diameter. The 1 × 10^6 cell inoculum formed a thin 5-mm diameter tumor at 1 and 3

**Figure 3.** Treatment temperature variation. Treatment temperatures >49°C are most effective in the treatment of 591 murine melanoma tumors.

**Figure 4.** Treatment time variation. Duration of treatment time between 10 and 60 seconds at 51°C does not influence effectiveness.

**Figure 5.** Tumor volume variation. Hyperthermia treatment is most effective in thin melanoma tumors.
days postimplantation with a 15% recurrence rate (4/27 animals). At 5 and 7 days postimplantation, the tumors continued to grow horizontally to 6 mm diameter and a recurrence rate of 21% (6/28 animals) was noted. By day 9, the tumors had begun to increase in height as well as diameter. The mean height was 3 mm and the mean diameter was 7 mm. By this time it appears that the tumor had grown to the maximal height that the hyperthermia device could reliably treat. We found a 13% recurrence rate (2/15 animals). Tumors at day 11 postimplantation had a mean height of 4 mm with an 8 mm diameter, thereby exceeding the depth penetration of the desired 52°C treatment temperature. There was a 40% recurrence rate (6/15 animals). We still noted a response to treatment with tumors that were 4 mm thick as heating occurred at that depth; however, based on our temperature measurement data, it is likely that the desired treatment temperature of 52°C was not attained at a 4 mm depth. We were probably heating the 4 mm deep tumor cells to 48°C, which is below the 49°C temperature we have demonstrated to be effective for these small tumors at a treatment duration of 30 seconds.

DISCUSSION

We have demonstrated that 2 mHz radiofrequency hyperthermia is effective in the treatment of cutaneous murine melanoma. It appears that higher temperature heating may be the optimum treatment strategy for small tumors, thus allowing for maximal tumor kill while minimizing treatment duration. Storm and colleagues have previously shown that tumors subjected to radiofrequency heating have a higher temperature than surrounding normal tissue due to decreased heat dissipation [19]. A shorter treatment duration at temperatures above 42°C is possible since an increase of a single degree of temperature will decrease the exposure time by a factor of two to achieve the same amount of cell killing [20]. It is not known, however, how this relationship varies for temperatures as high as 50–52°C. Other hyperthermic treatment strategies include: high intensity ultrasound at temperatures of 43–45°C for 30 minutes [21], microwave at temperatures of 42–43.5°C for 45 minutes [22], and water bath at 42.5°C for 30–120 minutes [23]. These long heating periods become cumbersome when multiple sites require treatment.

Radiofrequency hyperthermia has been successfully used by others in the treatment of malignancy. Dickson and colleagues treated rabbit hind limb intramuscular VX2 carcinomas with 13.56 mHz radiofrequency hyperthermia noting complete regression in 7 out of 10 tumors [24]. Grier and coworkers used 2 mHz radiofrequency hyperthermia to treat 45 ocular squamous cell carcinomas in cattle and horses resulting in 80% complete and 16% partial regression [25]. Sugara and associates treated 3 lung cancer patients with 13.56 mHz radiofrequency prior to surgical removal of the tumorous lung and noted massive tumor necrosis [26]. We have added to this experience by demonstrating the utility of radiofrequency hyperthermia in murine melanoma. Some of the limitations of this technology, however, were illustrated by our data. We were only able to successfully treat 7-mm-diameter tumors under 4 mm in depth. The depth limitation is advantageous in that it prohibits production of a third degree burn, but it also does not allow adequate treatment of thick tumor nodules. The diameter limitation can be overcome by overlapping treatment fields. Our recurrences were probably based on inadequate depth penetration and inadequate overlapping of treatment fields.

Many tumor treatment modalities with both chemotherapy and radiation therapy are effective at killing malignant cells, but are themselves carcinogenic. Hyperthermia has never been shown to be carcinogenic and is only weakly mutagenic [27]. Therefore, hyperthermia does not induce tumors as a result of treatment. Hyperthermia can easily be combined with other cancer treatment modalities to increase tumor kill. Heating and radiation can be combined additively since hyperthermia damages hypoxic cells preferentially and radiation preferentially kills well oxygenated cells [28–30]. Hyperthermia can also be used prior to surgery to decrease tumor load or even to treat the field surrounding the tumor to destroy microscopic foci of tumor [31]. Lastly, hyperthermia can be combined with chemotherapy to augment tumor kill [32]. We are currently investigating these strategies in our laboratory.

In summary, we have demonstrated the following: (1) Capacitive radiofrequency hyperthermia is effective in the treatment of small murine S91 melanoma tumors. (2) Duration of treatment between 10 and 60 seconds at 52°C does not influence effectiveness in small tumors. (3) Treatment temperatures >49°C are most effective in the treatment of small murine S91 melanoma tumors for a treatment duration of 30 seconds. Based on these preliminary findings, high-temperature short-duration capacitive radiofrequency hyperthermia deserves more study in the treatment of cutaneous malignancies. Studies are currently in progress to assess fractionated doses at relatively lower temperatures in an effort to design the optimal treatment protocol.

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