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Circulatory Responses of Malignant Tumors during Hyperthermia

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The use of hyperthermia (elevation of regional body temperature to 41.5°–45°) as an adjuvant to clinical radiation therapy is becoming accepted in clinical practice at this time. It is, therefore, imperative to define the physiological responses of tumors to this modality. In this article, the effect of hyperthermia on the physiological responses of human and murine tumors are evaluated employing pH, oxygen, and flow ultramicroelectrodes. It is determined that hyperthermia causes a rise in tissue oxygen tension (T_pO_2) and blood flow at temperatures up to 41°, with a decrease at higher temperatures. Tumor tissue pH is low (6.8) and decreases during hyperthermia by as much as one unit of pH. The evidence linking these observations and the importance of blood flow modifications are discussed.

INTRODUCTION

A great number of studies have demonstrated that hyperthermia retards the growth rate of certain types of malignant tumors with minimal damage to normal tissues (Suit, 1977).

A possible explanation for the differential response to heat that exists in tumorous tissue in relation to normal tissue could be provided by a better understanding of the physiological microenvironment of the tumors as it differs from the microenvironment of the normal tissues. For example, several studies indicate that the pH of fluid in human and rodent solid tumors is lower than that of the normal tissue pH of 7.4 (Eden, 1955, Gullino, 1965, Meyer, 1948, and Nawslund, 1953).

Several other parameters may change and subsequently influence the response of cells or tissues to supranormal temperatures. Paramount among those are the vascular changes, blood flow responses, and the net result of this on tissue oxygenation that may change the effect of both hyperthermia and radiation therapy when used in combination therapy.

However, insufficient information is available on the effect of temperature modifications on tumor blood supply, tumor oxygen tension, and consumption and respiratory gas exchange by malignant cells under *in vivo* conditions.

In a previous, preliminary publication (Bicher 1978), the effect of localized, microwave-induced hyperthermia on normal brain T_pO_2 (tissue oxygen tension) levels and the ability of the microcirculation to deliver O_2 to tissue upon breathing

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the gas were described. Tissue oxygenation was markedly improved in both cases when tissue temperature was raised up to 41°. Similar preliminary results were also reported in tumors (Bicher, 1980).

Hyperthermia is rapidly becoming an active modality in the treatment of cancer. With a view to further understand its mechanism of action, an evaluation of the physiological responses to hyperthermia in normal and tumorous tissues using microelectrodes to measure T_pO_2, pH, and local blood flow in tumors *in situ*, both in animals and man, was undertaken.

MATERIALS AND METHODS

1. Tumor Systems

Measurements were performed on two different tumor systems, as follows.

(a) C_3H mouse mammary adenocarcinoma. In situ studies were carried out in fourth-generation transplants of C_3H mammary adenocarcinoma implanted in the hind leg of C_3H SED-BH mice. The tumors were obtained from the Radiobiology Division, Massachusets General Hospital (Suit, 1978). This is a syngeneic implantable tumor that is kept at our facility using solid tissue transplants that are inoculated subcutaneously into recipient mice. Tumors used for experimentation were approximately 10 mm in diameter. The mice were anesthetized during microelectrode introduction with a combination of Ketamine 40 μg/kg im and Thorazine, 50 mg/kg im.

(b) Human tumors. Determinations were made in subcutaneous metastases in a group of 15 patients. Tumors represented different histologies and locations, but are grouped together as the responses were homogenous. There were four melanomas, six chest wall recurrences of mammary adenocarcinomas, and five peripheral metastases of squamous cell carcinoma of the lung. The patients were not anesthetized. Oxygen was administered through a facial mask when required (see below-oxygen ultramicroelectrodes).

2. Physiological Determinations

(a) Oxygen ultramicroelectrodes. The O_2 ultramicroelectrodes used were of the "gold-in-glass" type as described by Cater and colleagues (1959). They were made by pulling a glass tube (KG-33, i.d. 1.5 mm, o.d. 2.0 mm, Garner Glass Co., Claremont, Calif.), encasing a 20-μm gold wire (Sigmund Cohn Corp., Mt. Vernon, N.Y.) in a David Kopf Model 700C vertical pipet puller. The exposed gold tip is about 10 μm in diameter, and is coated with a Rhoplex (Rhom Haas, Pa.) membrane as previously described (Bicher, 1978). This probe is used as an "external reference" O_2 microelectrode. The electronic circuitry to measure the polarographic current was provided by a Model 1200 Chemical Microsensor System (Transdyne General Corp. Ann Arbor, Mich.), and the results were recorded on a Grass Model 7 polygraph. The procedure for electrode calibration was the same as previously described by Bicher (1970).

In human experiments, a platinum–iridium Teflon-coated wire, 120 μm in diameter, was used as the O_2 electrode. Although the calibration was not as reliable to determine actual T_pO_2 values, it was found in determining transients (responses to oxygen breathing or hyperthermia) that the obtained values corre-

lated well with those obtained using microelectrodes. The responses to O_2 breathing were determined by administering pure oxygen to the mouse or the patient for 1 min. The height of the tissue oxygen response provided an indication of the ability of the circulation to transport oxygen, probably dependent on the blood flow. The temperature artifact of both types of oxygen electrodes was determined and found to be 5% per degree celsius. All results were corrected by taking this artifact into account.

(b) pH ultramicroelectrodes. Tissue pH was measured using a 1-μm tip glass microelectrode constructed according to the Hinke (1978) technique. Basically this sensor consists of a small exposed area of pH-sensitive glass protruding from a micropippet made of pH-insensitive glass which acts both as carrier and insulation. The pH microelectrodes are connected to a Transdyne MPA-6 amplifier leading to a DC channel in the Grass polygraph.

(c) Microflow. Flow in microareas of tumor tissue was determined using the hydrogen diffusion method as described by Stosseck and Lubbers (1974). The method is based on the polarographic determination of the amount of hydrogen gas reaching a surface platinum electrode from a hydrogen-generating electrode located at a fixed distance. The amount of hydrogen reaching the reading electrode depends on the generation and diffusion rates, which are constant, and the blood flow clearance of hydrogen, which can be thus determined. In the present experiments two platinum in Teflon 100-μm wires placed 100 μm apart were used. The reading device was applied to the surface of the tumor. In the present experiments only relative changes in the rate of blood flow were determined. This method was used in the experiments on mouse tumors *in situ* (Materials and Methods 1a).

(d) Temperature determinations. Tumor and mouse core temperatures were recorded using Copper–Constantan microthermocouples (tip diameter 30–100 μm) inserted into the tumoral tissue in close proximity to the O_2 microelectrode or in the animal's rectum for core measurements. An Omega Engineering Model 250 digital voltmeter amplifier was used as a link between the microthermocouple and the polygraph. Microwave of a frequency 2450 MHz were produced by a Raytheon magnetron and delivered through a specially designed 5-cm diameter circularly polarized applicator loaded with low-loss dielectric material having a dielectric constant of 6 (Sandhu, 1978).

The use of relatively large fields compared to the size of the tumor allowed for uniform heating of the tumors (± 0.5°). With proper alignment the presence of the electrode caused no modification in the heating pattern.

RESULTS

Similar results were obtained in all tumors *in situ*, both in mouse mammary and human tumors, as localized microwave hyperthermia was applied to reach progressively higher temperature levels. Results were as follows.

TpO_2. As can be seen in Fig. 1, there is a rise in TpO_2 that parallels the application of the microwaves and closely follows changes in tissue temperature. The response is very fast with TpO_2 increasing shortly after the rise in temperature, and then decreasing as the tumor cools off. This effect was present when heating was carried out up to 41°. At higher temperatures there was an initial

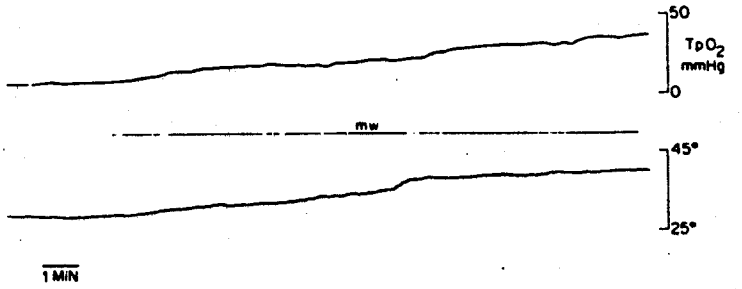

FIG. 1. Simultaneous records of TpO_2 (upper trace) and tissue temperature (lower trace). Microwave (mw) are applied during the time shown by the central solid line.

increase in TpO_2 which was followed by a decrease to very low levels as the temperature was held constant at 46° (see Fig. 2).

Local blood flow. Figures 3 and 4 show examples of the effect of hyperthermia on local blood flow in mice. In both cases it is clear that blood flow increases significantly up to approximately 41°. In addition, examination of the data in Fig. 4 shows the strong correlation between decreases in TpO_2 and blood flow as the temperature is increased up to 45°.

Tissue pH. The mean value of tissue pH was found to be 6.8 pH units in mouse tumors. Upon heating for 1 hr at 43°, there was a pH decrease of 0.5 to 1 pH unit to an average of 6.2 (Fig. 5).

Response to O_2 breathing. Breathing O_2 for 1 min usually causes a very small rise in TpO_2. Local hyperthermia caused an increase in this response that was proportional to the local tumor tissue temperature. The threshold was about 37.5° and up to 40–50 mm Hg increase could be recorded at 41° (Fig. 6). This effect was also reversed when the tumor was heated to 45° (as seen for TpO_2 alone—for example, see Fig. 4).

DISCUSSION

The present studies clearly demonstrate that localized microwave hyperthermia causes a rise in tumor TpO_2 and blood flow up to 41°, with a fall at higher temperatures, while pH decreases markedly. The mechanism of this effect seems to be predominantly mediated through the blood flow changes, the metabolic effects being secondary to a microcirculation that is activated at moderate hyperthermic temperatures (up to 41°) and damaged at higher temperatures.

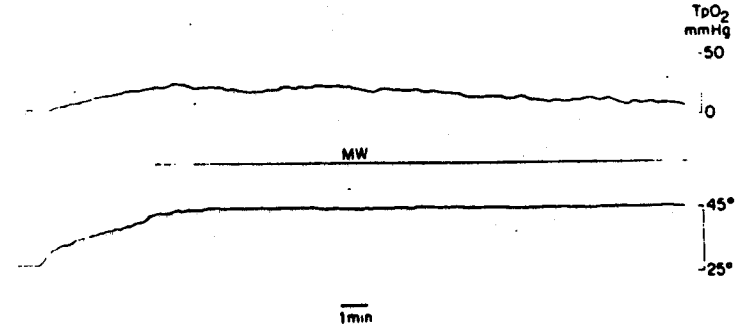

FIG. 2. Captions as in Fig. 1 except that temperature is raised to 45° in C_3H mouse tumor system.

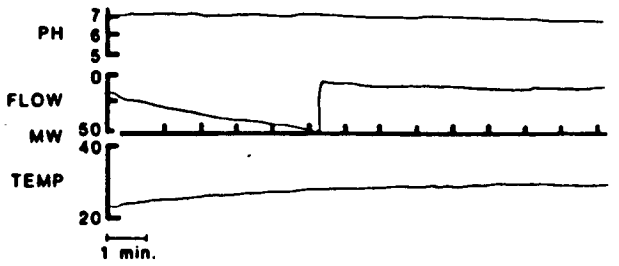

FIG. 3. Effect of microwave heating (microwaves on indicated by LLLLL) on tumor pH and blood flow. Blood flow tracing increased off scale and was manually adjusted about 5 min after heating commenced.

The rise in the tumor temperature up to 41° leads to a significant increase in tumor blood flow (TBF). This effect has also been demonstrated by England (1974) and Sutton (1976) for both the tumore region and host organ. As to the cause of this increased flow, presumably different factors have to be taken into account. The oxygen partial pressures in several subcutaneous tumors in animals and in humans as measured with 100-μm tip floating O_2 electrodes followed the change in blood flow (Bicher, 1980).

A further rise in tissue temperature up to 42° results in a marked breakdown of tumor blood flow to somewhat below the initial value. Similar results are obtained for *in situ* tumors, both in humans and mice. It has been shown in metastatic lesions involving the skin, that increase in flow occurs due to elevations of temperature up to 40°. With tumor temperature elevated to 46°, the tissue oxygen tension in microareas of the tumor decreases following a drop in tumor blood flow. This correlates with Reinhold *et al.* (1980), who have shown that at 42° the center of a "sandwich" tumor became necrotic due to a decrease of tumor microcirculation at this temperature. These results, however, do not correspond with those of Song (1978). This author found that hyperthermia at 43° did not change circulation in tumors, but that it did increase in normal tissues.

The restriction in blood flow at 42° and the increase in total vascular resistance,

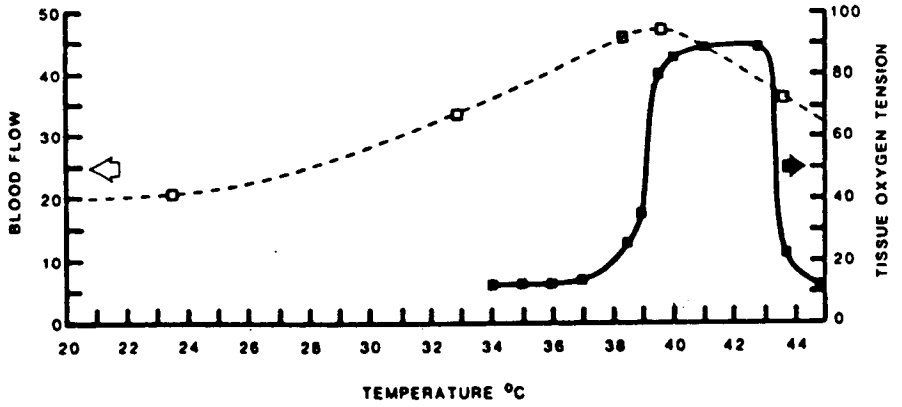

FIG. 4. Superposition of data obtained in a series of experiments in which blood flow and TpO_2 were determined at various temperatures in mouse tumors. The left axis and broken line are for blood flow while the right axis and solid line show TpO_2.

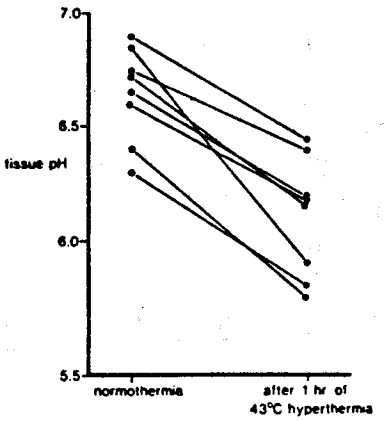

FIG. 5. Tumor tissue pH measured at several positions in mouse tumors before and after 43° hyperthermia. Connected data points show actual changes in pH at individual positions in the tumor since the microprobe was in place throughout treatment.

respectively, presumably result from a series of factors. As main determinants of the decline of blood flow, a reduction of red cell deformability, multiple microtherombae, as well as occlusions of microvessels have to be taken into account.

The results of a pH drop in the cancer tissue are not surprising if one considers the familiar principle that temperature strongly influences the buffering processes and hence the pH. There is usually a shift to lower pH values if the temperature is elevated. In addition, any increase in the CO_2 partial pressure, during hyperthermia, induced by changes in cellular metabolic pathways or activity would enhance tumor tissue acidosis.

As discussed earlier, a series of experiments conducted by several groups showed that elevation of the temperature within malignant tumors up to 41° leads to an increase in blood flow to a maximum in a certain temperature range. This maximum blood flow is accompanied by an increased oxygen and glucose consumption rate of the cancer tissue. From the given data, a series of consequences results for this temperature range of maximum tumor blood flow.

As the oxygen partial pressures in malignant tumors generally follow changes in blood flow, it can be expected that the radiosensitivity of cancer tissue may be improved during increased blood flow, thus producing a significant prolongation of survival time of tumor-bearing animals if they are treated with local hyperthermia in combination with radiation. Also, hyperthermia at higher temperatures (≥42°)

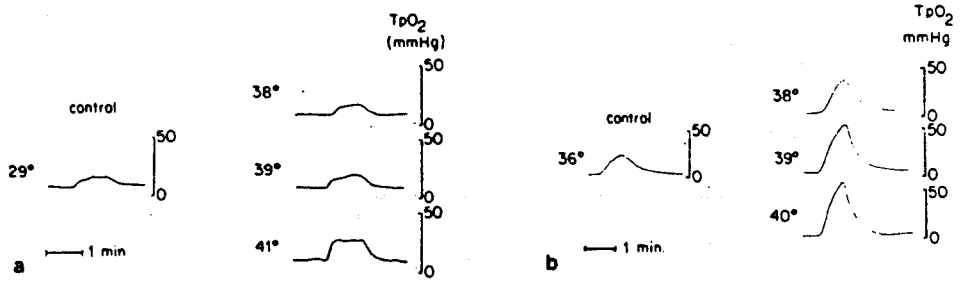

FIG. 6. TpO_2 is shown as a function of time during and immediately after 1 min of O_2 breathing while tumor is maintained at various temperatures, (a) mouse tumor, (b) human tumor.

would be most cytotoxic to those cells which were most radioresistant due to decreased pO_2, again increasing the combined effectiveness of the two modalities. Therefore, it is expected that hyperthermia can be a useful adjuvant during tumor therapy utilizing irradiation. This is supported by experiments showing that during combination of hyperthermia and irradiation, the survival time was longer than that during treatment with irradiation alone (Thrall, 1976).

Furthermore, it has to be taken into consideration that in the range of maximum tumor blood flow the convective transport of substrates, of wastes and what has to be stressed explicity, of antiproliferative agents may be improved. This improvement is of special interest since it can achieve higher concentrations of the antiproliferative agents in some tissue regions. In addition, by improving the substrate supply, a recruitment of the cancer cells belonging to the dormant G_0-fraction may be obtained, thus enhancing the cancerostatic effect.

We may conclude from the results presented here that the therapeutic effectiveness of hyperthermia may result, at least partially, from several induced physiological modifications. First, moderate (41°) hyperthermia in combination with ionizing radiation may result in improved tumor response by increasing oxygenation and hence, radiosensitivity coupled with a decrease in tumor pH. Second, higher levels of hyperthermia, 42° and above, may be directly tumorocidal because of an elimination of tumor micro blood flow and a concomitant sharp reduction in tumor pH.

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