13th International Cancer Congress, Part D
Research and Treatment, pages 235-245

IMPACT OF MICROCIRCULATION AND PHYSIOLOGIC CONSIDERATIONS ON CLINICAL HYPERTHERMIA

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The initial work of Thomlinson and Gray (1955) demonstrating necrotic regions in tumors at distances greater than 150 to 200 μ from a capillary and numerous reports of large hypoxic fractions in tumors (Bicher et.al. 1980; Kallman 1972) confirm that the microvascular network in tumors is poorly developed and organized when compared to that of normal tissues. It is this physiologic difference between tumor and normal tissue that may provide a therapeutic advantage for a modality such as hyperthermia.

Several general physiologic responses to hyperthermia have been commonly observed. These include increased cellular metabolic activity in the heated region and flushing of the skin overlying the heated area (indicative of increased perfusion of the skin). The specific responses in the microenvironment of different organs and tissues (malignant and nonmalignant) to modifications in temperature have been studied recently in more detail (Bicher, Mitagvaria, and Hetzel 1980; Reinhold, Blachiewic, and Berg-Blok 1979; Song 1978). Examination of several recent publications demonstrates that active investigations of the physiologic phenomena induced by hyperthermia are in progress (Bicher, Mitagvaria, and Hetzel 1980; Berg-Blok 1979; Storm et.al. 1979; Streffer er al. 1978; Von Ardenne and Reitnauer 1978). The studies by Eddy (1980) and by Reinhold, Blachiewicz and Berg Blok (1978) employing "chamber systems" of different types, have shown changes in the microvascular network as a function of temperature and exposure time. The apparent sensitivity of the neovasculature is a critical observation by these investigators. Similar results in different test

systems also have been observed by Emami and colleagues(1980) and by Dewhirst and Ozimek(1980).

Knowledge of the effect of hyperthermia on tumor and normal tissue blood flow and the subsequent effects on oxygen tension (pO_2) and pH is important not only for the effect of hyperthermia on hypoxic cells at the time of radition, but also for differential tumor heating.

There is considerable evidence from plethysmography that elevation of normal tissue temperature to 41°C is accompanied by considerable increase in blood flow (Lehman 1971). Cater and Silver(1960) reported on changes in tumor oxygen tension with hyperthermia but did not record changes in tumor temperature. They concluded that diathermy had not increased the oxygen tension in the tumor but on the contrary, caused a decrease.

Bicher and co-workers (1980) studying a mouse leg tumor system, reported that tumor blood flow increased at temperatures up to 41°C and then progressively decreased. The oxygen tension in the tumor, as measured with a platinum electrode, generally followed the changes in tumor flow, but the exact tumor temperature at which the oxygen tension decreased was not determined. Changes in tissue pH and brain tissue oxygenation were reported earlier by the same author (1978). The studies of Eddy(1980) and Reinhold(1980) employing "chamber systems" have both shown changes in microvascular network as a function of temperature and exposure time. Blood flow and pH changes during hyperthermia have also been reported by Song et al.(1980).

Although blood flow and shifts induced in it by hyperthermia in both tumor and normal tissue are important, several other parameters also have significant roles. Several studies indicate that the pH of interstitial fluid in human and rodent solid tumors is .3 to .5 units lower than the normal tissue pH of about 7.4.

Reduced pH has also been shown to affect the transplantability of tumor cells heated in vitro. In a recent paper,

**This work was partially reported at the 11th European Conference fro Microcirculation,Garmish, 1981(Bibl.Anat.N-20)and the ISOTT meeting in Detroit(1981). Thanks are given to Mr.S. Frinak for technical assistance in part of this work.

Gerweck(1976) has shown that there is a variable influence of pH according to temperature and that there is a critical point in the increased lethality of heat below pH 6.7.

In addition to hyperthermia, Hematopophyrin derivative (HpD) phototherapy is also showing some promise in clinical cancer therapy (Diamond et al. 1972, Dougherty et al. 1978). This type of therapy employs an injectable dye (HpD) which is specifically accumulated in some tumors and/or is specifically cleared from normal tissues. When light of specific wave lengths illuminate the dye of a photochemical reaction takes place yielding the cytoxic agent singlet oxygen (Weishaupt et al. 1976). Although the exact mechanism(s) of tumor inactiviation have yet to be determined, the rapid and dramatic coloration changes observed in tumors following treatment suggest that modification of blood flow with possible concomitant effects of pO_2 and pH may play a prominent role (Diamond et.al. 1972).

MATERIALS AND METHODS

Animal system. All in situ studies were carred out in 4th, generation transplants of C_3H mammary adenocarcinoma implanted in the hind leg of C_3H^3SED-BH mice. This is a syngentic implantable tumor inoculated subcutaneously into recepient mice. Tumors used for experimentation were approximately 8-10 mm in diameter. The mice were anesthetized during microelectrode induction with a combination of Ketamine 40mg/kg I.M. and Thorzine, 50mg/kg I.M.

Oxygen ultramicroelectrodes. The 0_2 ultramicroelectrodes used were as described by Cater and colleagues(1960) They were made by pulling a glass tube(KG-33, ID 1.5mm OD 2.0mm, Garner Glass Co., Claremont, California), encasing a 20-μ gold wire (Sigmund Cohn Corp., Mt. Bernon, New York) in a David Kopf Model 700 C vertical pipette puller. The exposed gold temp is about 10 μ in diameter, and is coated with a Rhoples(Rhom Haas, Philadelpia, Pa.) membrane as previously described (Bicher 1977). This probe is used as an "external reference" 0_2 microelectrode.

Electrodes are calibrated as previously described(Bicher et al. 1978) in buttered saline solutions of known pO_2 values. The electrodes are 'conditioned' by placing them in buffered saline and applying 0.8V potential for 2 hrs. After this treatment they are usually very stable. The current reading

at zero oxygen tension is very low (residual current) and the response of the microelectrode to changes of oxygen tension is very rapid.

In these experiments a polarizing voltage of 0.6V has been used. The relation between current output and oxygen tension is linear, the current mm Hg being of the order of magnitude of 0.6×10^{-11}A.

pH ultramicroelectrodes. Designs for glass pH microelectrodes employed in this study have been developed, most notably by Hinke(11). The Hinke-type electrode consist of a pH sensitive glass micrpipette inside of pyrex glass pipette with the tip of the pH sensitive micropipette extruded from the pyrex glass pipette. A silver/silver chloride electrode is inserted into the electrode with an exposed tip has an almost instantaneous response time. This is an advantage over the other types of microelectrodes in which a recessed tip may cause a response time of up to several minutes.

Temperature determinations. Tumor and mouse core temperatures were recorded using Copper 0 Constant and microtherocouples (tip diameter 30-100 micros-METRA Inc., Encino, CA) inserted into the tumoral tissue in close proximity to the 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 micro-thermocouple and the polygraph. Microwaves of a frequency 2450MHz were produced by a Raytheon Magnetron and delivered through a specially designed 2cm square applicator loaded with low loss dielectric material haveing a dielectric constant of 6.

HpD phototherapy. Tumor-bearing mice were injected with 20mg/kg HpD and 24 hrs later were exposed to the light source. The mice were shielded from the light during the 55 min. exposure except for the tumor-bearing region. The light source was modified Bessler latern slide projector which was filtered to yield 150,W/cm^2 at the tumor surface over the range 600-730 mm.

RESULTS

4 different major experiments were performed. In the first pO_2 distribution after 1 hr. hyperthermia was studied 1, 4 and 24 hrs after treatment. The second experiment was similar but studied the effect of Phototherapy on TpO_2, at

similar time intervals. Experiment 3 studied the effect of hyperthermia on tissue pH, at similar time intervals, while experiment 4 determined and compared the effect of Hyperthermia and phototherapy on tissue pH. All results were expressed in Histogram fashion

Experiment 1: Effect of hyperthermia on TpO_2-time correlation. Histograms were obtained before and 1, 2 4 and 24 hrs after microwave induced hyperthermia (42-43°C) for 1 hr. These results are shown in Figure 1. There is a progressive shift towards low pO_2 values, with all the tumor virtually hypoxic after 24 hrs. No reoxygenation is noted.

Experiment 2: Effect of Phototherapy on TpO_2-Time correlation. Histograms were obtained before and 1, 2 4 and 24 hours after HpD phototherapy (20mg/kg HpD, light, exposure 150mW/cm^2 for 55 min.). The pO_2 histogram shifts toward the hypoxic region (0.5mm Hg) quickly. However, at 4 hrs. there is an attempt to reoxygenate, as shown by the reappearance of an oxic tail in the 20-40mm Hg region. At 24 hrs. all values are between 0-10mm Hg. These results are shown in Figure 2.

Experiment 3: Effect of Hyperthermia on TpH. Time correlation. Histograms obtained before and 1, 4 and 24 hrs after microwave reduced Hyperthermia (42-43°C) for 1 hr. Results shown in fig. 3. Note a remarkable shift of the tissue pH values towards acidity with mean TpH changing from 6.74 to 6.21 after heat. TpH remains in that region at 4 and 24 hours.

Experiment 4: Effect of Hyperthermia and Phototherapy on Tissue pH-a correlation. Histograms obtained before 1 hr after microwave induced Hyperthermia, Phototherapy or a combination of both. Note that the pH shift towards acidosis (same as shown in fig. 3) induced by hyperthermia is not present after Phototherapy. This correlation is shown in figure 4.

DISCUSSION

Determination of the mode of tumor inactivation by a treatment modality is critical to its development and future use. It has been shown that hyperthermia may have possible effects on cell survival either alone or in combination with radiation (Gerwick 1977, Overgaard 1976, Rheinhold 1978). It has also been shown to dramatically modify blood flow and

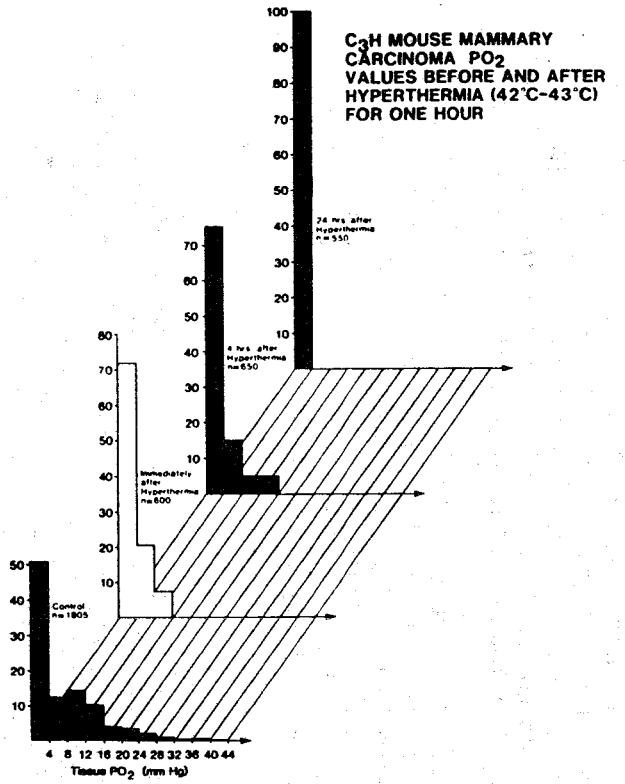

Figure 1. Effect of Hyperthermia on $Tp0_2$

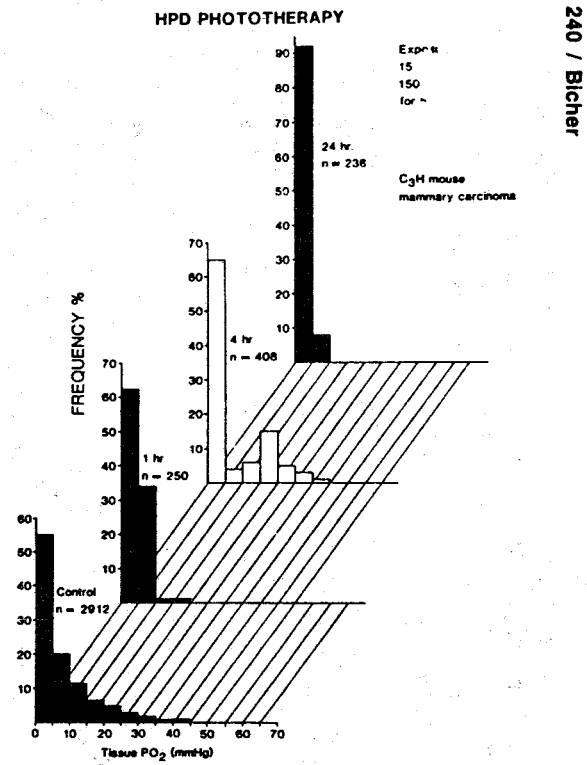

Figure 2. Effect of HpD Photo-therapy on $Tp0_2$

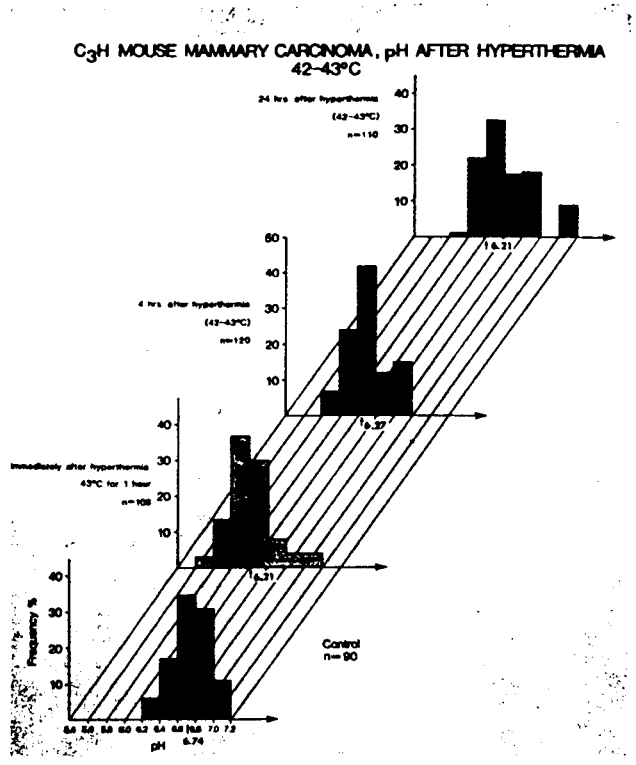

Figure 3. Effect of Hyperthermia on TpH.

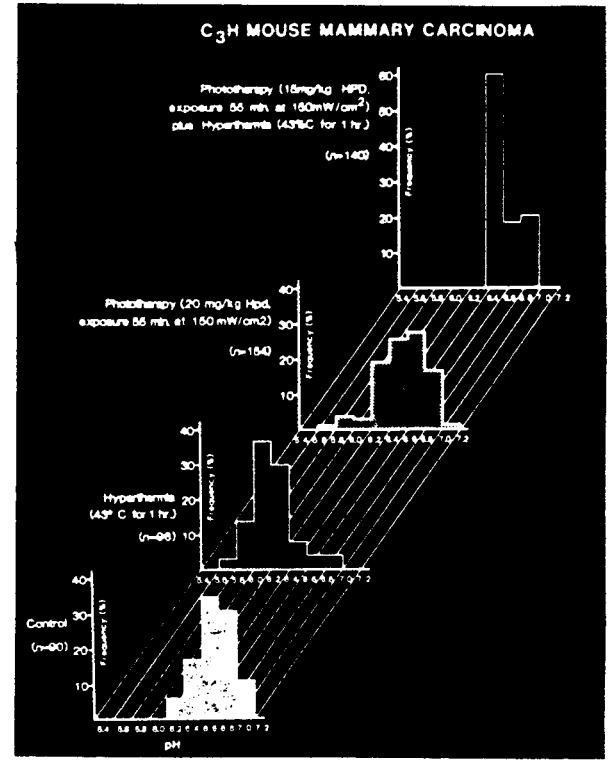

Figure 4. Effect of Phototherapy, Hyperthermia or a combination of both on TpH.

oxygenation within tumors (Bicher et al. 1980). The results presented here indicated that a significant reduction in pH is induced by hyperthermia which may result in a significant increase in cell killing with the tumor (Gerwick 1977). It is possible that this observed pH shift is due to a combination of hyperthermia stimulated cellular metabolic activity and the simutaneous reduction in tumor blood flow which is observed at the treatment temperature (Bicher et al. 1980).

The effects seen on pH and pO_2 following HpD phototheapy are quite different from those following hyperthermia. It is clear that ther is a sharp reduction in pO_2 at all areas within the tumor without any significant shift in pH. Although studies are now in progress, it is possible to speculate on the meaning of the results presented here. Massive coagulation necrosis within tumors is reported to follow HpD phototherapy (Diamond et al 1972). It is likely that the cells most affected by this treatment are the vascular endothelial cells of the tumor microvasculature. Their destruction would result in the observed coagulation necrosis with an abrupt reduction in blood flow. This would result in the low levels of tissue oxygenation reported here. Since there is no cellular metabolic stimulation with this modality and there is direct cytotoxicity (Weishaupt et al. 1976) no dramatic shift in pH would be expected and none was observed.

Further studies are currently in progress to further examine the effects of hyperthermia and HpD phototherapy on tumor microphysiology. Results so far are seen in figure 5.

MICROPHYSIOLOGY
C_3H Mice Adenocarcinoma Implanted in Leg Muscle
Hyperthermia and Phototherapy

1. O_2pH Inhomogeneities occur in tumor&muscle tissue
2. Important microcirculation changes occur in both Phototherapy & Hyperthermia.
3. One hour 43°C Hyperthermia:
   A) Collapses Microcirculation
   B) O_2Histogram shifts toward 0-5mm Hg O_2 Decrease with time
   C) No Reoxygenation up to 24 hours
   D) pH shifts toward Acidosis
4. Phototherapy: A) Microcirculation Damage
   B) O_2 Histogram shifts toward 0-5mm Hg quickly
   C) Reoxygenation at 4 hours
   D) No pH shift

SUMMARY

Changes in tumor tissue oxygenation and acidity were determined using ultramicroelectrodes, and presented in histogram fashion. The effect of Hyperthermia and HpD phototherapy were tested. It was found that both modalities affect tumor microcirculation, causing a marked drop in oxygen availability. Tissue pH is decreased by Hyperthermia, but not by phototherapy. These effects are long lasting at least for 24 hours after treatment.

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