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MICROCIRCULATION MODIFICATIONS BY LOCALIZED MICROWAVE HYPERTHERMIA AND HEMATOPORPHYRIN PHOTOTHERAPY*

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Introduction

Due, in part, to increasing clinical interest, active investigation of the physiological phenomena induced by hyperthermia is in progress (1,2,7,13). The studies by Eddy (7) and Reinhold (13) employing "chamber systems" have both shown changes in the microvascular network as a function of temperature and exposure time. Cater et al. (3) reported on changes in tumor oxygen tension with hyperthermia but did not record changes in tumor temperature. Bicher (1,2), in a mouse leg tumor system, reported that tumor blood flow increased up to 41°C and then decreased to 44°C. The oxygen tension in the tumor, as measured with a platinum electrode, generally followed the changes in tumor blood flow.

Although blood flow and shifts induced in it by hyperthermia in both tumor and normal tissue is 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 (8,10).

Reduced pH has also been shown to affect the transplantability of tumor cells heated in vitro (12). In a recent paper, Gerweck has shown (9) 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, Hematoporphyrin derivative (HpD) phototherapy is also showing some promise in clinical cancer therapy (5,6). This type of therapy employs an injectable dye (HpD) which is specifically accumulated in some tumors and/or is specifically cleared from normal tissues (5,6). When light of specific wavelengths illuminate the dye a photochemical reaction takes place yielding the cytotoxic agent

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singlet oxygen (7,15). Although the exact mechanism(s) of tumor inactivation 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 on pO_2 and pH may play a prominant role (5).

Materials and Methods

Animal system. All in situ studies were carried out in 4th., generation transplants of C_3H mammary adenocarcinoma implanted in the hind leg of C_3H SED-BH mice. This is a syngenetic 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 8-10mm in diameter. The mice were anesthetized during microelectrode introduction with a combination of Ketamine 40mg/kg I.M. and Thorazine, 50mg/kg I.M.

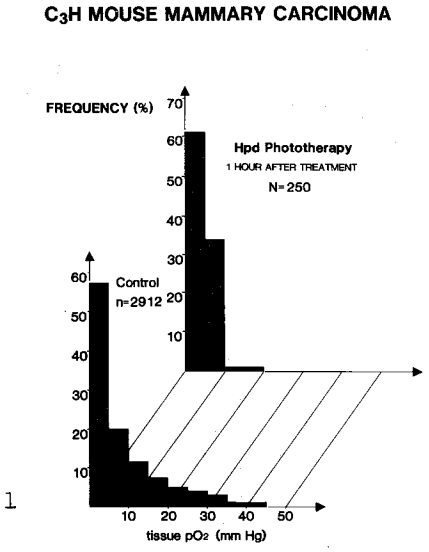
Oxygen ultramicroelectrodes. The O_2 ultramicroelectrodes used were as described by Cater and colleagues (4). They were made by pulling a glass tube (KG-33, ID 1.5mm, OD 2.0mm, Garner Glass Co., Claremont, California), encasing a 20-μ gold wire (Sigmund Cohn Corp., Mt. Vernon, New York) in a David Kopf Model 700C vertical pipette puller. The exposed gold tip is about 10μ in diameter, and is coated with a Rhoplex (Rhom Haas, Philadelphia, Pennsylvania) membrane as previously described (2). This probe is used as an "external reference" O_2 microelectrode.

Electrodes are calibrated as previously described (1) in buffered saline solutions of known pO_2 values. The electrodes are 'conditioned' by placing them in buffered saline and applying 0.8 V 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 0.6 V has been used. The relation between current output and oxygen tension is linear, the current per 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 consists of a pH sensitive glass micropipette inside a 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 stem which is filled with 0.1 N HCl. This type of microelectrode with an exposed tip has an almost instantaneous response time. This is an advantage over 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-Constantan microthermocouples (tip diameter 30-100 microns - MEDTRA Inc.) inserted



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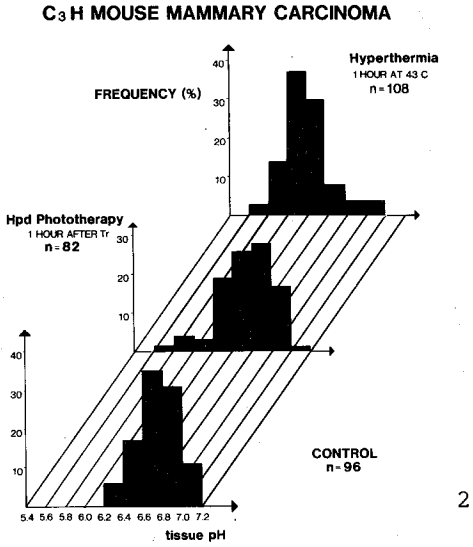

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Fig. 1. Oxygen histograms obtained in mouse tumors with microelectrodes.

Fig. 2. pH histograms following HpD phototherapy or hyperthermia treatment of mouse tumors.

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 microthermocouple and the polygraph. Microwaves of a frequency 2450 MHz were produced by a Raytheon Magnetron and delivered through a specially designed 2cm square applicator loaded with low loss dielectric material having a dielectric constant of 6 (14).

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

Results

Figure 1 shows the oxygen histogram obtained in mouse tumors 1 hour following phototherapy. It is clear that the normal pO_2 distribution is eliminated with almost all readings below 10mm Hg O_2. A similar trend is not seen, however, when the pH profiles are examined at the same time. In Figure 2 the pH histograms obtained in control tumors and in tumors 1

hour following either 43°C hyperthermia or HpD phototherapy are presented. In this case there is a dramatic shift in pH from a mean of 6.8 ± 0.2 in controls to 6.2 ± 0.2 following hyperthermia. There is also a trend to lower pH following phototherapy but the shift is not significant.

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 has many possible effects on cell survival either alone or in combination with radiation (9,12,13). It has also been shown to dramatically modify blood flow and oxygenation within tumors (1,2). The results presented here indicate that a significant reduction in pH is induced by hyperthermia which may result in a significant increase in cell killing within the tumor (9). It is possible that this observed pH shift is due to a combination of hyperthermia stimulated cellular metabolic activity and the simultaneous reduction in tumor blood flow which is observed at the treatment temperature (1).

The effects seen on pH and pO_2 following HpD phototherapy are quite different from those following hyperthermia. It is clear that there is a sharp reduction in pO_2 at all areas within the tumor without any significant shift in pH. Although further 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 (5). 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 (15) 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.

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