Thermal Biology and Physiology in Clinical Hyperthermia: Current Status and Future Needs

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Abstract

The current status and needs of thermal biology and physiology as related to clinical hyperthermia are summarized. Emphasis is placed on heat-induced modification of blood flow and microenvironment in tissues, on the biological effects of heat and X-rays, and on the relationship between drug resistance, heat resistance, and thermotolerance in thermochemotherapy. Results from recent studies investigating the relationships between thermotolerance and heat shock proteins in tissue culture cell lines, in rodent tumors, and in normal tissues are presented. These data strongly suggest that the level of M, 70,000 heat shock protein can be used as an assay to predict the thermal sensitivity of tissues during fractionated hyperthermia.

Introduction

Hyperthermia has been shown to potentiate the action of radiation and some cytotoxic drugs (7, 17). The use of hyperthermia alone or as an adjuvant to radiotherapy or chemotherapy is being investigated clinically for the treatment of cancer. The purpose of this manuscript is to provide a brief summary of the current status and needs of thermal biology and physiology as related to clinical hyperthermia. Emphasis is placed on the complexity and implications of heat-induced modification of blood flow and microenvironments in normal and malignant tissues; on the biological effects of heat and X-rays; and on the relationships between heat resistance, drug resistance, and thermotolerance in thermochemotherapy. I will present results from some recent studies investigating the causal relationships between the induction of thermotolerance and enhanced synthesis of a family of proteins, so-called HSP2. Three systems are examined: tissue culture cell lines; rodent normal tissues; and murine tumors. The data strongly suggest that the quantification of HSP 70 can be used as an assay to predict the thermal sensitivity and to determine the kinetics of the induction and decay of thermotolerance during fractionated hyperthermia. The principles derived from these studies may be applied to the clinic.

Modification of Blood Flow and Microenvironment in Tissues by Hyperthermia

Blood flow plays a critical role in determining our ability to reach therapeutic temperatures and therefore is also critical in establishing the effectiveness of hyperthermia, either alone or combined with radiation or chemotherapy. Elevated temperatures induce changes in the vasculature of normal as well as tumor tissues (9, 44). The responses of blood vessels (or blood flow) will then affect, via blood flow-associated heat dissipation, the thermal regulation and temperature distribution of the tissues during heating. In addition, alteration in blood flow will modify the tissue microenvironment, e.g., the pH, oxygen tension, and nutritional status (4, 38). The changes in microenvironment have an effect on the thermal sensitivity of the cells, inhibit their ability to recover from thermal damage, change the kinetics of thermotolerance, and affect their response to subsequent heat and/or X-ray challenges (11–18, 39–41). When heat is combined with chemotherapy, changes in blood flow can directly modify the drug delivery; additionally, changes in microenvironment can affect the drug cytotoxicity. These heat-induced alterations in vascular function, physical temperature distribution, and biological microenvironment eventually affect the fate of the tumor and normal tissues within the treatment target volume. Over the past decade, considerable information has been accumulated on the effect of hyperthermia on blood flow and microenvironment, both in tumor and in normal tissues. Several excellent reviews have discussed this in great detail (4, 9, 41, 44).

Tumor blood flow and tumor vasculature are different from that of normal tissue. Inside the same tumor, the distribution of vasculature and the blood flow are heterogeneous and complex. In general, tumor blood flow appears to be less than that in corresponding normal tissues, although ample exceptions exist, particularly in small tumors (9, 44). When the normal tissue is heated, (42–45°), Song et al. (44) reported that the macroscopic measurements of blood flow in normal tissues show dynamic changes. The changes are rapid, usually accompanied by vasodilation and an increase in permeability of the vascular wall. The degree of change depends on the temperature and duration of the treatment. By contrast, the heat-induced changes in tumor blood flow and tumor vasculature are less significant when compared to normal tissues. In most of the animal studies, tumor blood flow either remained unchanged or increased slightly (usually less than a factor of 2). If the heating time was prolonged, most tumors showed a decrease in blood flow. Much of the work describing effects of heat on rodent vasculature and blood flow comes from Song's laboratory and is reviewed in this issue (43).

A similar conclusion can be drawn from microscopic measurements. Dudar and Jain (9) measured microvascular parameters, such as RBC velocity and vessel lumen diameter, in neoplastic tissue (VX2 carcinoma) and normal (mature granulation) tissues grown in transparent rabbit ear chamber preparations. They found that again, upon heating, normal tissue blood flow increased dramatically with temperature, but stasis occurred at high temperature (47°) and longer heating time (1 hr). In tumors, blood flow rate did not increase as much, and stasis occurred at a lower level of hyperthermia (41°, 1 hr) when compared to the normal tissues.

1 The abbreviations used are: HSP, heat shock proteins; HSP 70, M, 70,000 heat shock protein; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.
normal tissue measurement. The critical temperature was approximately 45.7°C for normal tissue and 43°C for tumor. The maximum increase in flow capacity was around 6-fold in normal tissue, but only 2-fold in tumors. They also observed that: (a) the relative peak blood flow was higher in normal tissue and reached maximum at 46°C; when heating temperature was greater than 46°C, the peak blood flow rate decreased; (b) time required to reach the peak depended on temperature of the heat treatment; at higher temperatures, longer time was required both in tumor and normal tissues; (c) time to reach stasis was sooner in tumor than in normal tissues.

Milligan et al. (32) developed a mathematical model using thermal clearance technique to estimate the regional blood flow during hyperthermia. They found that, when the limbs of mongrel dogs were heated with 2450-MHz microwave to temperatures of 43-47°C, the regional blood flow increased in normal tissue within the treatment volume. The increase in blood flow reached a maximum value and then decreased if the heating time was prolonged. Their data also indicated that the peak blood flow and the time required to reach the maximum depended on the temperature of the treatment. For comparison, Milligan et al. also studied the effect of 44°C hyperthermia on canine mast cell tumors. During heating at 44°C for 40 min, the tumor blood flow remained at the same level as that in the control animals. Interestingly, the investigators observed that, during fractionated hyperthermia (44°C, 40 min/treatment, 4 treatments over 9 days), the tumor tissue appeared to undergo changes and blood flow increased by a significant amount (2-fold). In contrast to tumor tissue, maximum blood flow within normal tissue increased only slightly. Olch et al. (34) observed an appreciable increase in blood flow in heated human tumors. It was difficult to raise the tumor temperature above 42°C in those cases, and the authors concluded that this was due to the effective cooling of the tumors due to the increased tumor blood flow. Studies on the effects of hyperthermia on blood flow of large animals and humans are few. Such data are certainly needed.

Due to the complex nature of tumor-normal tissue boundaries and the heterogeneous nature of tumor vasculature, the effects of heating on the spatial temperature distribution are far more complex than the macroscopic or microscopic physiological measurements can predict. Even if thermal energy were uniformly deposited, one should still expect heterogeneity in the spatial temperature distribution. Dewhirst et al. (8) used dogs and cats to study the radiation and heat response of spontaneous tumors. They showed that the temperature variation can vary up to 6°C within the same tumor. Furthermore, their data indicated that the coolest part of the tumor, or the minimum tumor temperature, governed the biological response to combined heat and radiation treatments.

Few data are available on the recovery kinetics of tumor blood flow after hyperthermia treatments. Steward and Begg (45) reported that after mild hyperthermia there was significant decrease in blood flow of mouse tumors 24 hr after heating and then a gradual recovery afterwards. Kang et al. (24) measured changes in blood volume in SCK tumors of A/J mice as a function of time after heating at 43.5°C for 30 min. The tumor blood flow dropped to about 10% of control value 3 to 5 hr after heating, remained low about 24 hr, and partially recovered by 48 hr. The effect of hyperthermia on tumor blood flow of large animals and humans and their recovery kinetics after heating (if there is any) remain to be determined.

Song et al. (38, 39), Bicher et al. (4), and Vaupel et al. (49), found that the intrinsically low intratumor pH further decreased upon heating. This increase in acidity, decrease in nutritional supply, and changes in oxygen tension would modify the responses of the cells to heat, radiation, and drugs. Studies on the alteration in microenvironment of tumor versus normal surrounding tissues of large animals during heating, their early recovery kinetics and long-term response after hyperthermia, and their subsequent effect on tissue response to various types of treatments are needed.

Biological Effects of Heat and X-Ray

Reviews of the biological effects of heat, interaction of heat with X-rays, and the time sequencing of heat and X-rays were presented by Hall and Roisin-Towle (22), Dewey (6) and Emami et al. (10) at this conference. In summary, heat interacts with X-ray synergistically (7). Heat complements X-ray; the age response to heat complements the age response to X-ray killing (51). Heat inhibits repair of sublethal and potentially lethal X-ray damage (3, 14, 26). Heat killing correlates with heat radiosensitization (23). Thermotolerance induces tolerance to radiosensitization. On the other hand, the X-ray response of thermotolerant cells, thermal adapted cells, and heat-resistant cells is the same when compared to the control cells (17). Work done by Warters and Roti Roti (50) and other investigators show that the cause of heat-induced radiosensitization may be due not to enhanced X-ray damage by heat but to repair inhibition. This inhibition of repair is probably due to alteration of substrate, but not the repair enzyme, so that damage was not recognized.

In vitro studies provide ample evidence that the time sequence for maximal heat-X-ray interactions occurs if the treatments are administered simultaneously. In animal studies, due to the heat-induced physiological changes, heating after X-irradiation may be more effective than the reversed order. Recently, Emami et al. (10) studied the effects of sequencing of the total course of 4 hyperthermia treatments (45°C, 15 min) and 8 fractions of irradiation (4000 rad) on local tumor control of murine RIF tumors. Their results showed that either 8 courses of X-ray treatment followed by 4 courses of hyperthermia or 4 courses of hyperthermia followed by 8 courses of X-ray treatment gave a 20% control rate. A superior control rate (70%) was achieved when X-ray treatment and hyperthermia were given close to each other. Interestingly, whether the combined heat and X-ray treatments were given at the first 4 courses, or the last 4 courses, or intermittently resulted in the almost identical tumor control rate of 70%.

Drug Resistance, Heat Resistance, and Thermotolerance in Thermochemotherapy

One of the promising approaches to the clinical application of hyperthermia is to combine it with chemotherapy ("thermochemotherapy"). Numerous in vitro and in vivo systems have been used to demonstrate the synergistic effects of hyperthermia when combined with a wide range of chemotherapeutic agents, such as Adriamycin, bleomycin, cyclophosphamide, nitrosourea,
The effectiveness of simultaneous administration of hyperthermia enhanced drug toxicity with hyperthermia (19, 29, 30, 36). To our knowledge, previous investigators have used cell lines relatively sensitive to Adriamycin alone, to demonstrate enhanced drug toxicity with hyperthermia (19, 29, 30, 36). The role of drug resistance needs to be carefully assessed when chemotherapy is combined with hyperthermia. We have tested the effectiveness of simultaneous administration of hyperthermia and Adriamycin in a cell line that was selected for resistance to Adriamycin. We found that in Adriamycin-resistant cells hyperthermia does not potentiate drug toxicity beyond that which can be achieved with heat or Adriamycin treatment alone (Chart 2). Drug resistance did not, however, confer heat resistance or X-ray resistance (Chart 3). We have also examined the cytotoxic effect of Adriamycin in 2 heat-resistant variants that were selected through repetitive heatings from their heat-sensitive parents (25). As shown in Chart 4, these heat-resistant strains demonstrated appreciable resistance to Adriamycin but not to X-irradiation. In contrast, Dewey (6) and others7 have shown that, when BCNU-resistant and BCNU-sensitive cells are exposed to BCNU at elevated temperatures, the resistant cells show more heat-induced sensitization to BCNU than do the sensitive cells. Again, in this case, drug resistance does not confer heat resistance. Recently, Hahn and Shiu (21) reported that lowering pH greatly enhanced the cytotoxicity at elevated temperature of some chemotherapeutic agents, such as BCNU. This finding certainly has clinical relevance because the interiors of many tumors are characterized by relatively low pH. What is the effect of pH and nutritional environment on the heat response and drug response of the intrinsically heat-resistant or drug-resistant cells? Will the acidic tumor environment also enhance the cytotoxicity of drugs at elevated temperature to some tumor cells that are heat and/or drug resistant and that may already have adapted to low pH environment? Studies to address these questions are certainly needed.

Thermotolerance and HSP Synthesis in Rodent Tumors and Normal Tissues: HSP 70, a Possible Predictor for Thermal Response

The last topic I will discuss is the relationship between the development of thermotolerance and the synthesis of HSP, and...
the possibility of using HSP 70 as a predictor of thermal response of tissues, both normal and malignant. Results from 3 systems are cited: tissue culture cell lines; rat normal tissues; and murine tumors.

HSPs are a family of proteins the synthesis of which is either induced or greatly enhanced by heat shock or other environmental stresses (2). This group of proteins is induced in a wide variety of biological systems ranging from Drosophila to yeast to mammalian systems (2, 28, 31), and it has been suggested that their function is related to thermal tolerance in Drosophila, yeast, and mammalian cells (28, 31, 33). Many investigators (28, 47) have performed experiments to determine the relationship of thermotolerance and HSP synthesis. Thus far, almost all results show a good temporal relationship between the HSP synthesis and thermotolerance development in many different mammalian cell systems.

Recently, we have examined the quantitative relationship between cell survivals and levels of HSP in thermotolerant cells and in stable heat-resistant variants. In Chart 5, survivals of exponentially growing, thermotolerant Chinese hamster ovary cells and their heat-resistant variants all exposed to 45° for 45 min are plotted as a function of the concentration of HSP, both constitutively present and/or induced by heat shock. These results show a good correlation between levels of HSP 70 and thermal sensitivity. When the concentration of HSP 70 decreases, the cell survival after a 45°, 45-min heat treatment decreases. These data indicate that the level of HSP 70 appears to be a good predictor of thermal response.

Thermotolerance can be induced in some rodent normal tissues and in certain tumors (12, 17, 48). Even though different end points were used in these studies, and the experimental design in some of the studies did not allow for differentiation between recovery from heat-induced sublethal damage and thermotolerance, the results from the in vivo studies were qualitatively similar to those from in vitro studies. Information on the kinetics of induction and disappearance of thermotolerance in tumors and surrounding normal tissues is essential when designing treatment protocols of fractionated hyperthermia in can-
Chart 5. Relationship between cell survival and levels of HSP in thermotolerant HA-1 cells and their stable heat-resistant variants. Survival of exponentially growing, thermotolerant HA-1 cells and of heat-resistant cells (strains 3011 and 2422) exposed to 45°C for 45 min are plotted against levels of HSP, both induced and constitutive. The levels of HSP are expressed as percentage of total [35S]-methionine-labeled proteins. To measure the total accumulated levels of HSP, cells were labeled continuously throughout the experiments. Open symbols, unheated HA-1, 3011, and 2422 cells; solid symbols, transiently thermotolerant HA-1 cells. Cells were made thermotolerant by exposure to 45°C for 15 min, followed by 37°C incubation for 8, 23, 24, 48, 72, or 96 hr. Data of Li."

![Chart 5](chart5.png)

Table 1

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No. of rats in experiment: 3 3 4 4 1 2 1 2 2

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The synthesis and accumulation of M, 70,000 protein in tissues of 6-week-old rats after 42°C hyperthermia (5)

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N. F. Mivechi and G. C. Li. Induction of thermal resistance and heat shock protein synthesis in murine bone marrow cells after mild hyperthermia, manuscript in preparation.
time before being sacrificed. Tumors were then removed, and tumor cell suspensions were prepared. Tumor cells were either challenged with the second heat treatment at 45°C or labeled with [3S]methionine at 37°C. The tumor response to the combined heat treatments was measured using the in vivo-in vitro cloning assay. The cellular proteins were analyzed by 1- or 2-dimensional gel electrophoresis. We found that mild heat shock induced thermotolerance in murine tumors (Chart 6), results consistent with those of others (48). The mild heat shock also enhanced the rate of synthesis and accumulation of some HSP, especially the Mr 70,000 HSP (Figs. 1 and 2).

In Table 2, we present the synthesis, accumulation of HSP 70, and expression of thermotolerance in SCC VII/SF tumors after a 43°C, 15-min heat treatment. The data indicate that the levels or amount of HSP 70 correlates well with thermotolerance. It appears likely that the measurement of levels of the HSP 70 can be used as an assay to determine the thermal sensitivity of tissues during fractionated hyperthermia as applied in the clinic.

Acknowledgments

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References


Fig. 1. Autoradiogram of a sodium dodecyl sulfate-polyacrylamide slab gel of [3S]methionine-labeled proteins showing the enhanced synthesis of M, 68,000, 70,000, and 87,000 HSP in murine tumors after an initial exposure at 41.5° for 40 min. C, unheated control; abscissa, recovery time (hr) in situ before labeling; right ordinate, molecular weights (x10^-3). A, actin (M, 43,000). Data of Li and Mak.

Fig. 2. Increased synthesis of HSP 70 in murine tumor after 42° hyperthermia. Tumor cells were labeled with [35S]methionine for 4 hr. Total cellular extracts were analyzed by isoelectric focusing in the first dimension (left to right), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis in the second dimension (top to bottom). A, actin; V, vimentin. Arrow, M, 70,000 proteins induced in heated and control unheated cells.
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