Hypothermia in Cancer Therapy: The Biological Basis and Unresolved Questions

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This article considers the biological basis of hypothermia in cancer therapy. More specifically, it focuses on research observations which suggest that hypothermia is especially damaging to tumor tissue versus normal tissue, and it suggests new directions for research which may be useful for predicting and maximizing this difference.

It is unlikely that hypothermia alone will be useful for the permanent local control of tumors. Virtually all clinical studies (reviewed in References 41 and 57) use hypothermia in combination, primarily with radiation but also with chemotherapy. Although hypothermia interacts synergistically with radiation and some drugs when the agents are applied simultaneously, it has not been established that this synergism is greater in tumor tissue than in normal tissue. For this reason and others, hypothermia and radiation are usually applied with a time interval of a few hours or longer between the two forms of treatment. Within this context, it is believed that each agent is preferentially effective against different cell populations within the same tumor.

Research dealing with the biological effects of 41-45°C hypothermia on mammalian cells has substantially increased during the past 10 years (21, 29, 45, 58). Several studies indicate that, although the intrinsic thermal sensitivity of cells varies somewhat among cell lines (52), this variability is not closely linked to a cell’s normal/malignant status (22). The rationale for hypothermia in cancer therapy is therefore accessory rather than intrinsic to the transformed state.

The Biological Rationale for Hypothermia in Cancer Therapy

Tumor Vasculature and Differential Heating. The growth and continued expansion of a tumor depend upon the development of neovasculature which differs from normal vasculature in various ways (10, 20, 49, 66). Tumor neovasculature develops from preexisting normal vessels and morphologically resembles large tortuous capillaries. The vessels are deficient or devoid of smooth muscle and pericytes. Hypovascularized regions within a tumor are not uncommon. Normal vessels incorporated in tumors may become elongated and tortuous, and arteriovenous anastomoses are apparent, especially at the interface of tumor and normal tissues. Secondary to these vascular irregularities, substantial regional variations in blood flow occur within a tumor (49). Tumor blood flow may also vary with tumor size: in rodent tumors, a substantial decrease in blood flow per g of tissue has been observed with increasing tumor volume (49, 55).

Normal tissue perfusion rates vary substantially and are organ or tissue type dependent. Table 1 indicates resting blood flow rates for several normal tissues and tumors in humans. In general, tumor blood flow rates lie between those of resting peripheral tissues and several highly perfused organs. Only a few measurements of blood flow rates or ratios in tumor versus homologous normal tissue have been reported (Table 1). In one of these studies, the blood flow rates in normal tissues were reported to be 2.5 to 30 times greater than the tumor flow rates (55). These pronounced differences were not observed in breast or liver tumors in comparison to their corresponding normal tissue (3, 51). The reported higher rate of blood flow through mammary tumor compared to normal breast (3) is not surprising in view of the high fat content of normal breast.

Blood flow plays a major role in heat dissipation, especially in large well-perfused tissue masses, as illustrated in Chart 1. In identical tissues exposed to the same heat input, the steady-state temperature increase is inversely proportional to the ratio of blood flow (48). In small tissue masses, heat loss via thermal diffusion becomes prominent, especially at very low blood flow rates (28). Clearly, the temperature rise in a tissue cannot be predicted on the basis of energy input only. Small differences in tissue temperature may be expected to have a major impact on tissue response to hypothermia, as shown in Chart 2. Temperature variations as small as 2°C maintained for 30 min alter the fraction of cells killed by a factor of more than 100.

Several devices and methods have been developed for the local and regional deposition of heat in tissue. Many of these techniques utilize nonionizing radiative techniques in which surface tissues are within the radiation field. Even with superficial skin cooling, fat and muscle may be the dose-limiting normal tissue due to their limited perfusion. However, blood flow in muscle (and skin) increases markedly with temperature. Studies in rats indicate that muscle and skin blood flow may increase by up to 5- or 10-fold or more at temperatures of 42-44°C, with little change in tumor blood flow (8, 56). Increases in blood flow of this magnitude in human skin, muscle, and fat may permit the differential heating of some tumors or tumor regions when heat is deposited equally in tumor and normal tissue.

Table 1 and Charts 1 and 2 illustrate the biological factors which must be considered in detail when preferential heating of tumors based on blood flow is used as a rationale for hypothermia. The blood flow of the overlying and adjacent normal tissue must be greater than the flow in tumor tissue, if energy is deposited uniformly in both tissues. If the blood flow of a particular normal tissue is found to be relatively high and constant among patients, the differential heating of some lesions may be
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Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Resting blood flow (ml/100 g/min)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>1.6</td>
<td>48</td>
</tr>
<tr>
<td>Fat</td>
<td>3.3</td>
<td>48</td>
</tr>
<tr>
<td>Skin</td>
<td>5.0</td>
<td>48</td>
</tr>
<tr>
<td>White matter</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td>Liver</td>
<td>28-30</td>
<td>48, 51</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>35</td>
<td>48</td>
</tr>
<tr>
<td>Gray matter</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>Kidney-cortex</td>
<td>400</td>
<td>48</td>
</tr>
<tr>
<td>Kidney-medulla</td>
<td>20</td>
<td>48</td>
</tr>
<tr>
<td>Thyroid</td>
<td>400</td>
<td>48</td>
</tr>
<tr>
<td>Adrenal</td>
<td>500</td>
<td>48</td>
</tr>
<tr>
<td>Normal breast</td>
<td>3.96 ± 0.99^a</td>
<td>3</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>18.78 ± 9.67</td>
<td>3</td>
</tr>
<tr>
<td>Carcinoma of liver</td>
<td>12.21 ± 5.83</td>
<td>51</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>38.4 ± 14.3</td>
<td>39</td>
</tr>
<tr>
<td>Anaplastic carcinoma</td>
<td>11.4 ± 5.1</td>
<td>39</td>
</tr>
<tr>
<td>Differentiated malignant tumors</td>
<td>13.7 ± 9.0</td>
<td>39</td>
</tr>
</tbody>
</table>

^a Mean ± SD.

Normal:tumor ratio.

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Chart 1. Effect of blood flow on temperature increase. The heat input is 0.1 W/g. The specific heat and density are 4.2 J/g°C and 1 g/cu cm, respectively (48).

anticipated. However, variations in blood flow within tumors and at the normal/tumor tissue margin will preclude uniform tumor heating (6). Hyperthermia will therefore probably be most effectively applied in combination with other forms of therapy.

**pH and Thermal Sensitivity.** The second major rationale for hyperthermia in cancer is based on the low pH of tumors compared to normal tissues. Low pH may occur secondary to an elevated rate of glycolysis which is commonly exhibited by tumor tissues under both aerobic and anaerobic conditions (1, 67). The metabolism of one mol of glucose via the glycolytic pathway (under steady-state conditions) leads to the net production of 2 mol of H+. In contrast, the oxidative metabolism of glucose does not result in net H+ production (25).

At least 6 separate studies have demonstrated that, although regional variations exist, the average and extreme extracellular pH values are lower in tumor than in normal tissue; i.e., an average pH of 6.99 ± 0.14 (SD) was observed in 23 different rodent tumors, whereas the pH was 7.36 ± 0.17 in 5 normal tissues (7, 26, 31). These relatively large pH differences have not been observed in all rodent tissues, however. The average pH in the neuroectodermal TVIA rat tumor was only 0.1 units lower than the pH of normal rat brain tissue (26). Only a few determinations of tumor and normal tissue pH have been made in humans. In these studies, as in rodents, the average pH in various tumors was lower than the pH in normal tissue (by approximately 0.3 to 0.4 pH unit) (2, 47, 63).

The effect of low pH on the sensitivity of cells to hyperthermia is illustrated in Chart 3. Reduction in the pH of extracellular medium below 7.0 results in a marked reduction in the fraction of cells surviving 42°C heat treatment. In general, the magnitude of the pH-sensitizing effects is most pronounced in the 41-43°C temperature range and decreases at higher temperature (13). Moderate treatment temperatures (41-43°C) are also of interest, as they are relatively nontoxic at the pH of normal tissue.

The effect of induced pH changes on tumor thermal sensitivity has been examined in transplantable rat tumors. High-dose glucose injection reduced tumor extracellular pH without affecting the pH of various normal tissues. In these studies, heat treatment plus hyperglycemia was significantly more damaging than heat treatment alone (7, 27). In humans, selective reduction in tumor pH has also been observed following glucose injection. In 8 of 9 patients receiving a 100-g glucose infusion, the average electrode pH in 8 melanomas decreased from 6.8 prior to infusion to 6.5 after infusion (2). Although tumor glycolysis probably plays the key role in tumor acidosis, tumor blood flow, i.e., nutrient supply and catabolite clearance, may also be important. Relevant to this possibility are studies involving the effect of glucose and the non- or poorly metabolized sugar, galactose. High-dose intraperitoneal injection of either glucose or galactose suppresses tumor blood flow; however, glucose has a substantially more pronounced effect on tumor pH (7). Apparently, galactose inhibition of tumor blood flow simultaneously affects a reduction not only in lactate clearance but also in glucose availability, which
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Chart 3. Influence of pH on the thermal sensitivity of Chinese hamster ovary cells (13). Bars, SD.

limits lactic acid production. It therefore appears likely that the rate of cell glycolysis is a major factor in the development of tissue acidosis.

Energy Status and Thermal Sensitivity. Under conditions of extreme nutrient deprivation, cell death occurs rapidly at 37°C (14). Nutrient deprivation probably gives rise to the necrotic foci which are observed in tumors as the radial distance from functioning capillaries increases (23, 60). Between the nutrient-rich capillaries and the necrotic zone, viable cells reside within a gradient of decreasing oxygen and glucose concentration. Decreasing oxygen and glucose concentration prolongs the cell generation time and causes a redistribution of cells in their cycle (32). As a consequence, the toxicity of cell-cycle specific drugs or other agents may be substantially modified. In addition, local tissue hypoxia may severely limit the toxicity of drugs the effect of which is mediated by activated oxygen species.

The presence or absence of either glucose or oxygen alone has little effect on thermal sensitivity (14, 31). However, when the supply of both nutrients is sufficiently reduced to bring about a reduction in intracellular ATP levels, thermal sensitivity is markedly enhanced, as shown in Chart 4. Chart 4 and related studies (42) show that cellular energy status is a major determinant of cellular thermal sensitivity under in vitro conditions. Little information is available regarding the energy distribution of cells within tumors. Nevertheless, the in vitro studies suggest that hyperthermia will be effective against the energy-deprived non-cycling tumor cell population.

Unresolved Questions and New Directions

Blood Flow and Differential Heating. A systematic and detailed evaluation of the blood flow rates in treated tissues is of fundamental importance to the use of hyperthermia. Imaging techniques such as positron emission tomography hold promise for the noninvasive measurement of tissue blood flow. Positron emission tomography is used to determine the concentration of positron-emitting isotopes within a transaxial slice of the body. With the use of specific isotopes, regional blood flow can be evaluated. This technique has been used to measure blood flow in human mammary tumors and normal breast (3).

A second promising area of research is based on the smooth muscle deficiency of tumor vessels and their reactivity to vasoactive drugs (40). The direct-acting vasodilator hydralazine has been shown to effect a redistribution of blood flow from tumor to surrounding tissue in a transplantable canine tumor model (65). This blood flow redistribution resulted in the selective heating of tumor tissue upon the local application of energy. In view of the significance of blood flow in tissue heating, additional studies dealing with the differences in tumor and normal tissue vasculature and perfusion are warranted.
**pH and Thermal Sensitivity.** Substantial uncertainty exists regarding the relative roles of glycolysis and blood flow in the development of tumor acidosis. However, it is clear that low blood flow is not the sole cause of low tissue pH, as several normal tissues have blood flow rates that are substantially lower (Table 1) than those of acidic human tumors. Also, in normal tissue such as exercised muscle, a significant increase in blood flow does not prevent the development of lactate acidosis secondary to elevated glycolysis. Similarly, a substantial decrease in tumor blood flow, as occurs following galactose injection, only minimally affects tumor pH (7). It therefore appears likely that the rate of glycolysis is a major factor in the development of tissue acidosis.

Studies to date indicate that the naturally occurring differences in tumor and normal tissue pH are significant and may be further enhanced. pH not only affects hyperthermic sensitivity but also may be expected to influence the cellular transport, metabolism, and cytotoxicity of chemotherapeutic drugs. A significant potentiation of the antitumor action of 5-fluorouracil in rats bearing the Flexner-Jobling carcinoma is produced by the intraperitoneal administration of large doses of glucose (33). Equivalent galactose injections had no effect on tumor response to 5-fluorouracil. At above-normal temperatures (41.5°C), the cytotoxicity of cy-clophosphamide in the murine FSa-II tumor is potentiated by injections of glucose (62). Under in vitro conditions, variation in extracellular pH has been shown to strongly modify the toxicity of several drugs, including 5-fluorouracil, Adriamycin, bleomycin, and 1,3-bis(2-chloroethyl)-1-nitrosourea (4, 22, 43, 64). A more detailed knowledge of the pH distribution in tumors should positively influence the development and use of pH-active chemotherapeutics.

The mechanism of pH sensitization to elevated temperature is poorly understood. Uncertainty exists regarding the relationship betwen the change in extracellular pH, intracellular pH, and thermal sensitization. Many but not all studies (e.g., Refs. 18 and 19) show that reduction in extracellular pH results in a reduction of intracellular pH. However, in general, an intracellular-extracellular pH gradient is maintained, except under toxic or metabolically stressful conditions (18). Knowledge of the site of pH sensitization is essential for the development of methods for enhancing thermal sensitivity by pH reduction. Relevant to this topic is the observation that an inhibitor of lactate transport strongly sensitizes cells to hyperthermia (30). Although this suggests an intracellular site for pH sensitization, drug sensitization to heat was also influenced by variation in extracellular pH.

A major difficulty in assessing the role and impact of glycolysis and pH on tissue thermal sensitivity is the problem of nondestructive assessment of phosphate metabolites, not only in experimental animal studies but also in patients (12). Recent developments in NMR coil design permit the nontoxic, noninvasive evaluation of high-energy phosphate profiles in relatively small tissue volumes (44). A sample NMR spectrum is shown in Chart 5. These spectra were obtained from 6.5- and 7.5-mm murine fibrosarcomas grown in the mouse foot. The relative concentration of each phosphate metabolite is obtained by integration under the peaks of interest. Each of the 3 phosphates of ATP (ATPα, β, and γ) gives rise to a distinct peak. The ATP-β peak is essentially free of other metabolites and is used for the calculation of ATP. For the data shown, the ratio of adenosine triphosphate to inorganic phosphate (ATP/Pi) is clearly smaller in the large (214 cu mm) tumor than in the smaller tumor and is indicative of a reduced aggregate cellular energy status. Intracellular pH is also obtained from the 31P-NMR spectrum from the shift in the resonance frequency of inorganic phosphate relative to a standard, e.g., phosphocreatine. For the data shown in Chart 5, the shift in the resonance frequency of Pi indicates only a slightly lower aggregate intracellular pH (=0.1 units) in the larger compared to the smaller tumor.

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Substantial changes in phosphate profiles have been observed following heat, radiation, or drug treatment of rat tumors (44). These changes indicate that 31P-NMR may be useful (at least in a qualitative manner) for evaluating treatment cytotoxicity (44). Of substantial interest is the possibility that the energy status of a cell (naturally occurring or modified) is predictive of tissue thermal sensitivity. For example, the spectrum shown in Chart 5 indicates that cellular energy status and intracellular pH are decreased in the larger FSa-II tumors. Chart 6 shows that the...

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2 L. E. Gerweck, unpublished observations.
3 The abbreviation used is: NMR, nuclear magnetic resonance.
treatment time required for permanent local control of FSa-ll tumors slightly decreases with increasing tumor size. Along with the in vitro energy status studies, the data in Charts 5 and 6 suggest that a relationship exists between tumor pH and/or energy status and tumor thermal sensitivity. If further study indicates that these metabolite levels are predictive of tissue thermal sensitivity, NMR may prove to be a valuable tool in the selection of lesions for treatment by hyperthermia.

Decay of Thermotolerance. The sensitivity of cells (and tissues) to hyperthermia is transiently but markedly reduced following an initial heat treatment (9, 24, 46). This resistance, which has been termed "thermotolerance," is expressed in cells which survive very mild to severe heat treatments. The time required for the development of maximum thermotolerance varies somewhat with the magnitude of the initial heat treatment, from a few hours following a very mild initial heat treatment to 10 to 15 h following severe initial treatments. Thermotolerance protects cells against hyperthermia not only with respect to the maintenance of their reproductive ability but also with respect to general cell metabolism (5, 53).

The development of thermotolerance appears to be related to a group of proteins called heat shock proteins. Following heat treatment, these proteins are synthesized at an increased rate simultaneously with the development of thermotolerance. This correlation has been observed in cultured cell lines (34, 37, 59) and in rodent tumor and bone marrow cells following in vivo heating (36). Low pH and energy deprivation have been shown to suppress thermotolerance development; however, significant differences in the kinetics of thermotolerance development in rodent tumor and normal tissue have not been observed (9, 24, 38, 46). This is not surprising, since thermotolerance is expressed in cells which survive the initial heat treatment. These surviving cells would predominantly arise from the tumor population which is most resistant to hyperthermia, i.e., from cells which are relatively well-nourished and at normal pH.

In contrast to the relatively similar and rapid rates of thermotolerance development, thermotolerance decay rates are slow and variable. Following identical or isoeffect initial heat treatment, thermotolerance decay rates varied by a factor of 3 between proliferating V-79 hamster cells and human glioblastoma cells (16). Thermotolerance decay rates may also be influenced by a cell's proliferative rate or status (15). Since thermotolerance is a powerful modifier of hyperthermic sensitivity, variability in the kinetics of tolerance decay in tumor and normal tissues could markedly influence the response of these tissues to fractionated heat treatment. Furthermore, it is not unlikely that tolerance decay kinetics is tissue-specific and relatively constant for tissues such as muscle, nerve, gut epithelium, lung, etc. Knowledge of tissue tolerance decay kinetics could be of substantial assistance in designing hyperthermia fractionation protocols which minimize damage to heated normal tissues and perhaps maximize damage to tumor tissue.

Heat Delivery. The delivery of energy to tumors without unacceptable normal tissue heating is a necessary prerequisite for the clinical application of hyperthermia. Several methods and techniques have been used for clinical hyperthermia. These include whole-body and interstitial heating techniques as well as heating via various externally placed electromagnetic and ultrasound power sources. Acceptable but frequently less-than-ideal heating patterns can be obtained in superficial or surgically accessible tumors by the implantation of electrodes or microwave antennae. Plane wave ultrasound or microwave applicators are also commonly used for the treatment of superficial lesions. However, the intensity of these fields decreases exponentially with depth and precludes their use for the treatment of deep-seated lesions.

Focused ultrasound waves are capable of delivering highly focused energy to deep-seated tissue volumes of various dimensions (35). However, ultrasound waves do not propagate through air cavities and are reflected and absorbed by bone. Nevertheless, this modality holds promise for the controlled heating of several superficial and deep lesions. Its continued evaluation in a clinical setting is clearly indicated.

Of the several electromagnetic devices used for "deep" heating, two have been used sufficiently often (with adequate thermal mapping) to allow at least a preliminary evaluation of their heating characteristics. Heating patterns produced by an annular phased-array microwave device driven at 55 to 100 MHz and a concentric single-turn self-resonant coil driven at 13.56 MHz were compared in 22 patients with pelvic and abdominal tumors (17). Neither device successfully heated upper abdominal tumors due to inadequate penetration (concentric coil) or excessive systemic heating (phased array). However, the successful heating of deep pelvic tumors was frequently attained with the phased array.

In summary, currently available techniques are being used to produce adequate but not ideal heating patterns in superficial or accessible tumors and normal tissues. In several of these studies, the combination of hyperthermia with other forms of therapy appears to be clearly advantageous. The continued development and careful evaluation of methods for heating tissue is clearly necessary.

Summary

The properties of a cell population which characterize it as malignant also render that cell population sensitive to hyperther-
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References

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1 min 15 min 50 min

Fig. 1. Positron emission tomography images of liver and spleen (lower left) after intravenous injection of [¹⁵F]-2-deoxy-o-glucose. At 50 min after injection, the activity of label in the colon cancer metastasis to liver (arrow) markedly exceeds normal liver activity. From Reference 68. Reprinted with permission.
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