Effect of Local Hyperthermia on Blood Flow and Microenvironment: A Review¹

Chang W. Song

Department of Therapeutic Radiology, Radiation Biology Section, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Abstract

The blood flow in tumors varies considerably among different tumor types. Even in the same tumor, the distribution of vasculature and blood flow is quite heterogeneous. The tumor blood flow generally decreases as the tumors grow larger, owing partially to progressive deterioration of vascular beds and to the rapid growth of tumor cell population relative to vascular beds. Contrary to the general notion that blood flow is less in tumors than in normal tissues, blood flow in many tumors, particularly in small tumors, is actually greater than that in surrounding normal tissues at normothermic conditions. Compared to the normal tissue blood flow, however, the capacity of tumor blood flow to increase upon heating appears to be rather limited. Consequently, the heat dissipation by blood flow in tumors is slower than that in normal tissues, and thus the temperature of tumor rises higher than that in normal tissue during heating. Preferential heating of tumors, however, may not be achieved all the time because the relative blood perfusion in some tumors remains greater than that in the surrounding normal tissues despite the profound increase in normal tissue blood flow during heating.

The vasculature in tumor can be significantly damaged at temperatures which may alter but do not damage the vasculature of normal tissue. Upon heating, the intratumor environment becomes acidic, hypoxic, and nutritionally deprived due probably to vascular damage. Such a suboptimal environment in the heated tumors potentiates the response of tumor cells to hyperthermia, inhibits the repair of thermal damage, and also interferes with the development of thermal tolerance. The acidic environment also appears to potentiate the response of tumor cells to certain drugs at elevated temperatures. The changes in oxygenation of tumors and normal tissues caused by the changes in blood flow may have significant implications in the effectiveness of different sequences of hyperthermia and radiotherapy in the combined use of these two modalities. Changes in the distribution of drugs in tumors and normal tissues due to changes in blood flow will also determine the optimal use of hyperthermia in conjunction with chemotherapy.

Introduction

The rise in temperature of tissues during heating is largely dependent on the influx of heat from the external heat source and also on the efflux of heat through dissipation by the circulating blood (20, 45). Therefore, preferential heating and damage of tumor can be expected only if heat is preferentially delivered to the tumor or if heat dissipation by blood flow is slower in the tumors than in the surrounding normal tissues. It is now an established fact that an acidic and nutritionally deprived environment greatly increases the thermosensitivity of tissue, inhibits the recovery of tissue from thermal damage, and perhaps inhibits the development of thermotolerance (14, 16, 18, 21, 33, 34, 42). The acidity and the nutritional supply of a tumor are closely related to blood flow. Blood flow also affects the tissue PO₂, and thus the response of tissue to radiation. It should be pointed out that the supply and distribution of drugs in tissues are also largely dependent on the blood perfusion in the tissues. It is then apparent that blood flow plays the central role in determining the effectiveness of hyperthermia used alone or in conjunction with radiotherapy or chemotherapy. In this review, the role of blood flow in the temperature distribution in tumor and normal tissue is discussed. In addition, the implication of intratumor environmental factors, which are related to blood perfusion, on the response of tumor cells to heat used alone or in conjunction with other modalities is discussed.

Vascular Changes Induced by Hyperthermia

Normal Tissue. It is a well-known fact that heat induces a prompt increase in blood flow accompanied by dilation of vessels and an increase in permeability of the vascular wall in normal tissues (46). The degree of pathophysiological changes in the vascular system in normal tissue is, of course, dependent on temperature and duration of heating. An excess exposure of tissues to heat results in a breakdown of vasculature followed by necrosis of the tissues. Surprisingly, information on the vascular changes in normal tissues at temperatures commonly used in clinical hyperthermia, i.e., 41-45°, is scanty, while the cutaneous vascular changes at higher temperatures have been investigated (15, 41).

We have investigated the vascular changes in the skin and muscle of rodents at different time intervals after hyperthermia for varying lengths of time at 41-45° (35, 43-51). The blood flow in the skin of leg of SD² rats, measured by the radioactive microsphere method, was 7.1 ml/100 g/min (Chart 1), and it changed dynamically when the leg was heated with a water bath. The blood flow in the skin increased by factors of 4 and 6 upon heating at 43° for 60 and 120 min, respectively. At 44°, the skin blood flow rapidly increased to 88.9 ml/100 g/min within 30 min, which was about 12-fold the control value. The blood flow then progressively decreased when heating at 44° was continued, but it was still about 5 times greater than the control value at the end of 120 min heating. At 45°, the skin blood flow increased to 101.0 ml/100 g/min within 15 min of heating, which was about 15-fold the control value. The blood flow then began to decline rapidly reaching less than one-half of the control value at the end of heating for 120 min. The blood flow in the muscle of the leg of SD rats was 4.6 ml/100 g/min at ambient temperature.

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² The abbreviations used are: SD, Sprague-Dawley; BFR, blood flow ratio (blood flow in tumors/blood flow in tissues).
ture (Chart 1). As in the skin, a pronounced elevation of blood flow occurred in the muscle upon heating. The blood flow increased to 3.5- and 8.0-fold the control value upon heating for 60 min at 43 and 44°, respectively, and remained elevated at these levels until the termination of 2 hr heating. At 45°, the muscle blood flow increased by 9-fold in 30 min and then returned to control value at the end of 2 hr heating. Dickson and Calderwood (6) measured the heat-induced changes in blood flow in the skin and residual tissue of feet of Wistar rats by the ⁸⁶Rb extraction method. The blood flow in the skin and residual tissue of foot increased 20- and 10-fold, respectively, upon heating at 42° for 1 hr. The blood flow then started to decrease when the heating was continued beyond 1 hr heating, but it still remained 4 to 7 times greater than the control value. With the same ⁸⁶Rb extraction method, we observed that the blood flow in the skin and muscle of C3H mouse legs increased by 5- and 4-fold, respectively, when heated at 43.5° for 1 hr. The blood flow in the skin and muscle gradually diminished and returned to control value within 1-3 hr after heating but again increased slightly 24 hr after heating. Similar results were also reported by Stewart and Begg (52), who studied the heat-induced changes in blood flow in mouse skin (Chart 2). The blood flow, measured with ⁸⁶Rb extraction method, increased by about 3-fold when heated with a 42.5° water bath for 1 hr and remained elevated for 24 hr after the treatment. Duder and Jain (7) measured various microvascular parameters in the individual vessels with the use of transparent chambers implanted in rabbit ears. The blood flow in the normal (granulation) tissue increased by a factor of about 6 upon heating at 45.7° for 1 hr and returned to normal level after heating. Further prolongation of heating or elevation of temperature resulted in vascular stasis. Milligan et al. (31) reported that the blood flow in the muscle of dogs, measured by thermal dilution method, was 61.5 ml/100 g/min, which is about 12 times that in the rat muscle that we observed (Chart 1). When heated at 44° for 40 min, the blood flow in the dog muscle increased by a factor of about 2. It should be pointed out, however, that the vasculature in some normal tissues is rather vulnerable to heat, and thus the capacity of blood flow to increase upon heating may be limited. For example, histopathological study by Falk (12) indicated that vasculature in the mouse jejunum was exceptionally thermosensitive since heating for 1 hr at temperatures as low as 40.5° caused considerable damage, particularly in the inner layer of the jejunum.

It is of interest to note that the blood flow in the "normal tissues" adjacent to tumors is usually greater than that in the normal tissue remote from the tumors. For example, the blood flow in the skin and muscle adjacent to Walker tumor 256 in SD rats was about 2 times greater than that in the skin and muscle...
Tumor. An indisputable fact emerging from various experimental data obtained during the last several years is that the heat-induced change in the blood flow in tumors is considerably different from that in normal tissues. We (44-46) observed that the blood flow in Walker carcinoma 256 of SD rats remained unaltered during heating at 42° for 1 hr. However, when the heating was prolonged, the blood flow decreased progressively, and complete stasis occurred at 3 hr. In the SCK tumor of A/J mice, the blood volume was only one-tenth of the control value 5 to 12 hr after heating at 43.5° for 30 min, and the vascular bed appeared to be completely destroyed after heating at 44.5° for 30 min (Chart 6). In the BA-1112 sarcoma of rat, the blood flow was reduced by about 50% upon heating for 40 min at 41° or 30 min at 42° (6, 10). Heating at 43° or 44° caused a more pronounced and long-lasting vascular occlusion accompanied by a widespread rupture of blood vessels and hemorrhage.

In some types of tumors, blood flow slightly increases during heating at relatively low temperatures or before the vasculature is damaged by heating at relatively high temperatures. We measured the heat-induced change in blood flow in mammary adenocarcinoma 13762A of Fischer rats (Chart 4). The blood flow before heating was 9.5 ml/100 g/min in 0.2 to 0.5-g tumors and 5.2 ml/100 g/min in 1-g tumors. When heated at 43.5° for 1 hr, the blood flow increased to 11.2 and 9.5 ml/100 g/min in the smaller and larger tumors, respectively. The blood flow then decreased to less than 20% of control in both groups 1 to 16 hr after heating. Sutton (58) investigated the change in blood flow in ependymoblastoma of C57BL mice. It was observed that heating at 40-42° increased the blood flow by about 50% during the first 15 to 30 min. The blood flow then declined rapidly at 42° and more slowly at 40°. After 60 min of heating at 42°, all of the tumors showed a decrease in blood flow which could be

of normal legs. As shown in Chart 3, the blood flow in these tissues adjacent to tumor also significantly increased upon heating. The blood flow in the skin and muscle adjacent to the mammary adenocarcinoma 13762A in the leg of Fischer rats increased by factors of 7.5 and 3.5 when heated at 43.5° for 1 hr (Chart 4), and returned to almost control level 1 to 16 hr after heating. The blood flow in the muscle surrounding the SMT-2A tumor of Wistar rats increased by factors of 1.6 at 42° and 3.2 at 44° (39) (Chart 5). These increases were relatively smaller than those that we and others observed in other tissues, as described above.
attributed to occlusion of blood vessels. An increase in blood flow up to 2-fold was observed in 60% of DS carcinoma of rats when heated at 43° for 20 min or at 44° for 15 min by Vaupel et al. (60). This initial increase in blood flow was followed by a decrease when the heating was prolonged. In the rest of the tumors, the blood flow began to decline from the start of heating. Stewart and Begg (53) reported that the blood flow in SAFA tumor of mice increased by a factor of 2 in 30 min at 42.5°, but the blood flow started to decrease when heated longer, and it returned to control value by the end of 1 hr heating (Chart 2).

The blood flow in all 4 types of tumors which these investigators measured declined significantly below the control value 24 hr after heating and then began to recover to control values thereafter. We compared the response of blood flow in RIF-1 tumor, skin, and muscle of C3H mice to hyperthermia at 43.5°. The blood flow in tumors increased by a factor of about 1.8 when heated for 1 hr at 43.5°, while the blood flow in the normal tissues rose to a much greater degree as described in the previous section. The tumor blood flow then diminished to about 10% of control 3 and 5 hr after heating at 43.5° for 1 hr. Shrivastav et al. (42) reported that the blood flow in the mast cell tumor of dogs at normothermic conditions was 87.8 ml/100 g/min, and it remained at the same level during heating at 40° for 40 min while the blood flow in the adjacent muscle increased by a factor of 2. These authors also reported that the tumor tissue appeared to undergo compensated changes by a significant amount during fractionated heating over a 2-week treatment period. Oich et al. (32) locally heated human tumors with a magnetic-loop hyperthermia unit (Magnetrode) and measured the tumor blood flow by the 133Xe clearance method. Of the 12 tumors treated, 8 tumors showed an appreciable increase in blood flow during heating. Interestingly, it was impossible to raise the temperature above 42° in most of these 8 tumors. The investigators concluded that the increased blood flow effectively cooled the tumors, thereby preventing the rise in temperature. The effect of relatively higher temperatures, i.e., 42–45°, on human tumor blood flow remains to be studied, although vascular damage has been observed in human tumors after hyperthermic treatment (56).

A number of investigators measured various vascular parameters during heating in tumors grown in transparent chambers. Eddy (8) studied the vascular response to heat in squamous cell carcinoma grown in the cheek pouch chambers of hamsters. Whereas temporal vasoconstriction and minimal pathological alterations occurred upon heating at 41° for 30 min, vasodilation, petechiae, stasis, occasional thrombosis, and some endothelial degeneration occurred at 43°. When BA-1112 rhabdomyosarcoma grown in transparent chambers in rats were heated, the velocity of RBC steadily declined at 40–42° (11), and a permanent cessation of circulation and necrosis occurred upon heating at 42.5° for about 2 to 3 hr (36). One of the interesting phenomena observed in the heated tumors grown in the transparent chamber was that leukocytes stuck to the vessel walls of post-capillary and collecting venules in the tumors and impeded the flow of RBC (7, 11). The occlusion and stasis in the affected capillaries were frequently irreversible. Duder and Jain (7) noticed a retardation of blood flow at temperatures as low as 41° in the VX2 carcinoma grown in rabbit ear chambers. The blood flow in this tumor permanently ceased when heated at 42.5° or higher temperatures for 30 to 40 min. As mentioned above, the response of vasculature to heating varies considerably depending on tumor types. The temperature and duration of heating which causes vascular damage and deterioration of blood flow in various experimental tumors are summarized in Table 1.

**Modification of Heat-induced Vascular Changes**

Attempts have been made to modify the vascular responses in tumors and normal tissues (2, 62) to heat in order to selectively reduce the tumor blood flow. Voorhees and Babbs (62) studied the effect of vasoactive compounds on the heat-induced changes in blood flow in tumors and normal tissue. An i.v. injection of hydralazine (0.5 ml/kg) to dogs decreased the blood flow by one-half in the s.c. tumor implants and increased the blood flow in the underlying muscle by a factor of about 3. When hyperthermia was applied in conjunction with hydralazine, there was a further decrease in the tumor blood flow and an increase in the muscle blood flow. As a consequence, the muscle blood flow was 17

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**Table 1**

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Animal</th>
<th>Temperature</th>
<th>Time</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Walker carcinoma 256</td>
<td>Rat</td>
<td>&gt;43.0°</td>
<td>1 hr</td>
<td>Song et al. (46)</td>
</tr>
<tr>
<td>Walker carcinoma 256</td>
<td>Rat</td>
<td>&gt;43.0°</td>
<td>1 hr</td>
<td>Gullino et al. (19)</td>
</tr>
<tr>
<td>Yoshida sarcoma</td>
<td>Rat</td>
<td>42.0°</td>
<td>1 hr</td>
<td>Dickson and Calderwood (6)</td>
</tr>
<tr>
<td>BA-1112 rhabdomyoscaroma</td>
<td>Rat</td>
<td>42.5°</td>
<td>40 min</td>
<td>Enderich et al. (11)</td>
</tr>
<tr>
<td>BA-1112 rhabdomyoscaroma</td>
<td>Rat</td>
<td>40.0°</td>
<td>1 hr</td>
<td>Endrich et al. (11)</td>
</tr>
<tr>
<td>BA-1112 rhabdomyoscaroma</td>
<td>Rat</td>
<td>42.5°</td>
<td>2.5 hr</td>
<td>Rainbow et al. (36)</td>
</tr>
<tr>
<td>DS carcinoma</td>
<td>Rat</td>
<td>42.0°</td>
<td>30 min</td>
<td>Vaupel et al. (60)</td>
</tr>
<tr>
<td>1376Z2A carcinoma</td>
<td>Rat</td>
<td>43.5°</td>
<td>1 hr</td>
<td>Rappaport and Song (35)</td>
</tr>
<tr>
<td>SCK carcinoma</td>
<td>Mouse</td>
<td>40.5°</td>
<td>30 min</td>
<td>Song et al. (47)</td>
</tr>
<tr>
<td>RIF-1 tumor</td>
<td>Mouse</td>
<td>42.5°</td>
<td>1 hr</td>
<td>Song et al. a</td>
</tr>
<tr>
<td>Mammary carcinoma</td>
<td>Mouse</td>
<td>42.0°</td>
<td>40 min</td>
<td>Bicher et al. (3)</td>
</tr>
<tr>
<td>Ependymoblastoma</td>
<td>Mouse</td>
<td>42.0°</td>
<td>1 hr</td>
<td>Sutton (57)</td>
</tr>
<tr>
<td>SAFA tumor</td>
<td>Mouse</td>
<td>42.5°</td>
<td>1 hr</td>
<td>Stewart and Begg (52)</td>
</tr>
<tr>
<td>CAMT</td>
<td>Mouse</td>
<td>42.5°</td>
<td>1 hr</td>
<td>Stewart and Begg (52)</td>
</tr>
<tr>
<td>CANT</td>
<td>Mouse</td>
<td>42.5°</td>
<td>1 hr</td>
<td>Stewart and Begg (52)</td>
</tr>
<tr>
<td>SAF</td>
<td>Mouse</td>
<td>42.5°</td>
<td>1 hr</td>
<td>Stewart and Begg (52)</td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>Hamster</td>
<td>43.0°</td>
<td>30 min</td>
<td>Eddy (8)</td>
</tr>
<tr>
<td>VX2 carcinoma</td>
<td>Rabbit</td>
<td>42.5°</td>
<td>40 min</td>
<td>Duder and Jain (7)</td>
</tr>
</tbody>
</table>

a Presented at the Fourth International Symposium on Hyperthermic Oncology, July 2–6, 1984, Aarhus, Denmark.
Mechanisms of Heat-induced Vascular Changes

Previous studies with cutaneous tissues demonstrated that the increase in blood flow upon heating is caused, at least in part, by dilation of vessels and the recruitment of capillaries following stimulation by vasoactive compounds, such as bradykinin and/or histamine (15, 41). Elevated cardiac output as a response to a rise in blood temperature may also increase the blood flow in the heated tissues as well as in other tissues. The mechanisms for the increase in blood flow by different stress, i.e., radiation or chemicals, may be different, and it is conceivable that the mechanisms for the heat-induced blood flow may not be the same in different normal tissues.

In view of the morphological features of tumor vessels, it is not surprising that the response of vessels in tumors to heat is distinct from that in normal tissues. The mechanism of heat-induced changes in tumor blood flow is still obscure. The hastily formed capillaries in tumors are made of single-layered endothelial cells without an external coat of elastic basement membrane. Furthermore, the capillary wall is lined in part by tumor cells between the gaps of endothelial cells. Therefore, tumor cells are often in direct contact with circulating blood, and proliferation of these tumor cells into the lumen of capillaries and obstruction of the blood perfusion may account, in part, for the progressive deterioration of blood flow as the tumors grow. Tumor blood vessels are usually twisted, sharply bent with coil-like features, and extremely dilated. In fact, it is suggested that the tumor capillaries are maximally dilated to meet the demand for nutrients by the continuously proliferating tumor cells. Another characteristic of tumor vasculature is the presence of abundant sinusoidal openings. Unlike normal tissue, in which many capillaries are closed at ambient conditions, all functional capillaries in tumors are open and used at near capacity, even under normothermic conditions. It is conceivable then that, because the tumor vessels are maximally dilated even at ambient temperatures, the tumor blood flow would not increase at elevated temperatures. The slight increase in blood flow in some tumors during heating may be due to a mechanical or passive dilation of the vessels by the increased flow of blood into the tumors from the adjacent normal tissue. In some tumors, host vessels are incorporated into the tumor mass as the tumor progressively invades the surrounding normal tissues. Since the incorporated host vessels may respond to heat, it is probable that the increase in blood flow in some tumors by heat results, in part, from the increase in blood flow in the host vessels. An elevation of cardiac output during local heating may also cause an increase of blood flow in both the tumor and normal tissue.

The rather fragile capillaries of tumors, which are devoid of elastic basement membrane, may not allow complete vessels to cope with the great stress imposed by the elevated inflow of blood. In this connection, the thermosensitivity of endothelial cells is not known, although our preliminary studies with cultured endothelial cells appears to indicate that endothelial cells are relatively heat resistant. When endothelial cells or tumor cells, which are interdispersed in the tumor capillary wall, are damaged by heat, blood would inevitably leak out through the gaps. A sudden drop in blood pressure in the capillaries due to such hemorrhage may lead to vascular stasis. In addition to the rupture of the vascular wall, changes in the rheological properties of blood may also contribute to the occlusion of vessels in heated tumors. The rigidity of RBC membranes has been known to increase in an acidic milieu. As mentioned earlier, VonArdenne (61) suggested that RBC, coursing through the acidic tumor, lose their deformity at elevated temperatures, and the rigid RBC plug the capillaries. The increased adherence of leukocytes to vascular wall, particularly of venules, appears to be a common phenomenon in tissues after thermal injury as mentioned earlier (7, 11, 13). Such an accumulation of leukocytes on the inside of vessel walls would further obstruct the passage of deformed and rigid RBC.

We may then conclude that the heat-induced retardation of blood flow in tumors is due partly to the combined results of (a) swelling of endothelial cells, (b) lysis of endothelial cells and tumor cells in the capillary wall accompanied by leakage of blood, (c) sticking of leukocytes to vessel walls, and (d) an increase in rigidity of...
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RBC and viscosity of blood. Some of these changes may be enhanced by the acidic milieu in the heated tumors.

Relationship between Blood Flow and Tissue Temperature during Heating

Whether the tumors are preferentially heated and thus preferentially damaged by hyperthermia depends largely on the relative rate of heat dissipation by blood perfusion in the tumors and normal tissues. In Chart 7, the relative change in blood flow in tumors and that in the skin and muscle of rats upon heating at different temperatures are graphically summarized. It can be seen that, in most animal tumors, the blood perfusion deteriorates when heated for 30 to 60 min at 41–43°C. Such a vascular collapse occurs at temperatures as low as 41°C in some tumors (Table 1). In some experimental tumors, the blood flow increases before vascular collapse occurs upon heating. Usually, the increase in tumor blood flow is less than 2-fold. On the other hand, the blood flow in the skin and muscle of SD rats increases by a factor of 8 to 10 during heating at 43–45°C. As described earlier, blood flow in the skin of Wistar rats increased as much as 20 times at 43°C (6), and the blood vessels in some normal tissues remain intact at temperatures as high as 46°C (7). It should be pointed out that Chart 7 merely indicates the relative change in blood flow as a function of temperature, and it does not show the change in absolute values of the blood flow. We previously proposed the use of BFR to predict whether or not preferential heating of the tumor relative to the surrounding normal tissue can be expected (43). It has often been mentioned that tumors are poorly perfused or that the blood flow in tumors is poorer than that in normal tissues (28, 54, 55). Contrary to this previous notion, it is not uncommon to observe that the blood flow in tumors, particularly in the small tumors, is greater than that in surrounding normal tissues. As shown in Table 2 and Chart 3, the blood flow in the Walker tumor was larger than that in the surrounding muscle. Therefore, the BFR was >1. During the heating at 43°C, the BFR for the small Walker tumors was 1.95, while it was 0.49 for the large tumors. Thus, it would be reasonable to expect that the small tumors were more effectively cooled by blood perfusion than was the surrounding normal tissue, while the blood flow, and thus the heat dissipation in the large tumors, was only 49% of the blood flow in the normal tissues. In the case of SAFA tumor (Chart 2), the relative blood flow in the skin and tumor was almost the same (BFR = 1) at the beginning of heating. The BFR then became <1 after heating for 30 min by virtue of an increase in skin blood flow and decrease in tumor blood flow. Similar conclusions may be drawn for the 13762A tumor (Chart 4) heated with 43.5°C water bath. The core temperature of the 13762A tumors, larger than 1.0 g, was 42.3–43.0°C during heating, while that in the surrounding muscle was 41.5–42.4°C (35). It would be reasonable to attribute the lower muscle temperature to an efficient heat dissipation by the increased blood flow.

The clinical observations that temperatures in human tumors rise higher than that in the surrounding normal tissues (26, 28, 32, 54, 56, 58) may also be attributed to sluggish blood flow in the tumors relative to that in the normal tissues. However, blood flow in certain tumors would remain greater than that in the normal tissue, despite the greater degree of increase in normal tissue blood flow during heating. It would be difficult to raise the temperatures in such tumors higher than that in the normal tissues (32). It is conceivable that certain areas in a tumor are poorly vascularized, and thus the temperature in such areas rises higher than in the surrounding well-vascularized areas or normal tissues. The tumor cells in such poorly vascularized areas might be radiobiologically hypoxic, and thus radioresistant, while these cells may be selectively killed by hyperthermia owing to the preferential rise in temperature and also to the acidic environment associated with the hypoxic condition. Changes in BFR during the course of fractionated heatings as well as the effect of combined use of radiation or drugs with heat on the BFR remain to be elucidated.

Changes in Tissue pH and pO2 Caused by Hyperthermia

pH. It is now an established fact that the acidic environment greatly increases the thermal damage. It has long been known that the microenvironment in malignant tumors is intrinsically acidic relative to normal tissue due most probably to the preponderance of glycolytic metabolism, accompanied by formation of lactic acid. We (47) previously reported that the already acidic intratumor environment in SCK tumors of mice and Walker tumor 256 of rats became more acidic upon heating at 43–46°C. Recently, we further investigated the heat-induced changes in tissue pH using microelectrodes 50 to 80 μm in diameter (37). As shown in Charts 8 and 9, the intratumor pH in SCK tumors of A/J mice ranged from 6.6 to 7.3 with an average value of 6.92
before heating. When heated at 43.5° for 30 min, the average intratumor pH of SCK tumors immediately and significantly decreased to about 6.71 and then returned to control value 24 hr after heating. The pH in the leg muscle of mice was 7.42 before heating and did not change when heated at 43.5°. Bicher et al. (3) reported that the average pH in control C3H mammary tumor was 6.8 and that heating at 43° for 1 hr decreased the pH to 6.2. The recent data by Vaupel et al. (60) also showed that the intratumor pH in Yoshida sarcoma of rats was 6.89 before heating, and it dropped to 6.65 after heating for 60 min at 44°. The intratumor pH then recovered to 6.93 at 24 hr after heating.

In order to reveal the cause of the increase in acidity in the heated tumors, we determined the nature of acidic metabolites and observed that the lactic acid content in the SCK tumor significantly increased upon heating at 43.5° (40). Streffer et al. (55) reported that, not the lactic acid content, but the content of 3-β-hydroxybutyric acid increased in heated tumors. The pH in heated tumors remains low for a considerable length of time after heating, suggesting that the formation of acidic metabolites is continued after heating or that their excretion is hampered due to the decrease of blood flow.

\[ \text{pO}_2 \]

Whereas the tissue \text{pO}_2 itself may not directly affect the thermal effect, it has an indirect and significant impact on the thermal damage in tissues since the tissue acidity is closely related to the tissue \text{pO}_2. Bicher et al. (3) studied the effect of heat on the \text{pO}_2 in mammary carcinoma of C3H mice and in cat brain. The \text{pO}_2 in the tumor steadily increased upon heating until the temperature reached about 41° and rapidly declined at higher temperatures. Also in cat brain, the \text{pO}_2 increased as the temperature was increased and then rapidly decreased at temperatures above 43°. These investigators attributed the rise and fall of tissue \text{pO}_2 to a heat-induced increase and decrease in blood flow, respectively, and concluded that the critical temperature for the reduction in blood flow in the brain was higher than that in the tumor. Essentially the same conclusion was reached by Vaupel et al. (60), who estimated the oxygenation status of DS carcinoma of rats from the hemoglobin \text{O}_2 saturation value of RBC in the tumor capillaries. These investigators reported an increase in tumor oxygenation after heating for 30 min at 40°. At temperatures above 43°, however, the tumor oxygenation markedly declined, which was apparently due to a severe restriction of blood flow.

We quantitated the hypoxic cell fraction in SCK tumor with the use of a radiobiological method (51). About 45% of clonogenic cells in the unheated control tumors were radiobiologically hypoxic. The hypoxic cell fraction increased to about 95% at 5 hr after heating at 43° for 30 min, and then decreased to about 60% at 12 and 24 hr after heating. It should be emphasized that the increase in hypoxic cell fraction in tumors after heating does not imply that the absolute hypoxic cell number increases. Actually, despite the increase in the fraction of hypoxic cells, the absolute hypoxic cell number in SCK cells decreased drastically after heating at 43° for 30 min due to the decrease in the total number of surviving cells. Urano and Kahn (59) also observed a marked increase in hypoxic cell fraction in 2 tumor types of C3H mice 1 to 3 days after heating at 43.5° for 45 min. Undoubtedly, the above observations that the increase in hypoxic cell fraction and the decrease in \text{pO}_2 in tumors after hyperthermia resulted from deterioration of supply of oxygen owing to heat-induced vascular damage. Contrary to the above, the hypoxic cell fraction in tumors may decrease when the blood flow in the tumors is increased at relatively low temperatures.

Implication of Blood Flow, pH, and \text{pO}_2

The relationship between the tissue temperature and blood perfusion is already discussed above. The other aspects of the role of blood flow and also the implication of pH and \text{pO}_2 in the response of tumors and normal tissues to heat alone or in combination with other modalities are being elucidated by a number of investigators.

As shown in Chart 10, we observed that the slope (D₀) of the thermal survival curve of SCK tumor cells heated \textit{in vivo} at 42.5° was 32 min (25). On the other hand, the D₀ for \textit{in vitro} heating at 43° was 55.5 min, demonstrating that the thermosensitivity of tumor cells \textit{in vivo} was markedly greater than that \textit{in vitro}. Recently, Rofstad and Brustad (39) also reported that human melanoma cells grown in the immunodeficient nude mice were far more heat sensitive than were the same cells grown \textit{in vitro}. We concluded that the acidic and nutritionally deprived intratumor environment \textit{in vivo} rendered the tumor cells sensitive to heat (25).
Another intriguing phenomenon in the response of tumor cells to heating in vivo is the additional or further cell death after heating. We observed in SCK tumors that the clonogenic cell number progressively decreased for 5 hr when the tumors were left in situ after heating (Chart 11) (24, 25, 47). Similar additional death after heating was observed in EMT-6 tumors by Marmor et al. (30). In this context, Dewey et al. (5) reported that the number of surviving V79 cells progressively decreased when the cells were heated and maintained in acidic medium in vitro. It would then be reasonable to conclude that the enduring acidic, hypoxic, and nutritionally poor microenvironment as a result of vascular damage in heated tumors enhances the death of tumor cells, which could otherwise recover from the thermal damage. The above observations of the additional cell death in the tumors after heating are in agreement with a 2-decade-old observation by Crile (4). In this investigation, Sarcoma 180 of Swiss mice was heated in vivo and the transplantability of the tumor to new hosts at various times after heating was quantitated. The development of tumor in the new hosts was significantly reduced when the transplantation was done 8 hr after heating as compared to transplantation immediately after heating.

The use of hyperthermia in the treatment of neoplastic tissue is complicated by the development of thermotolerance (14, 23, 27). Although the mechanism underlying the development of thermotolerance is far from clear, indications are that the degree of development of thermotolerance is somewhat suppressed or delayed by suboptimal environment, in particular acidic conditions (2, 16, 18, 33, 34). Our recent investigations demonstrated that, at least in SCK tumor of AJ mice, not only was the development of thermotolerance delayed, but also the degree of the thermotolerance was reduced in vivo as compared with those in vitro (38). In fact, fractionated heating in 2 doses appeared to be more effective than a single heating for SCK tumors in vivo, provided that the fractionation interval was shorter than 5 hr. We postulated that the environment in the heated tumor, presumably the acidic, hypoxic, and poor nutritional condition, impeded the development of thermotolerance for 1-3 hr and also enhanced the response of the heated cells to subsequent heating. In view of the profound difference in the microenvironment in the heated tumors and normal tissues, it is tempting to speculate that the kinetics and magnitude of development of thermotolerance in tumors and normal tissues might be different. If this is the case, preferential damage in malignant tumors may be obtained by properly fractionating hyperthermic treatment. Further detailed study on the implication of heat-induced changes in vascular functions and microenvironment in tissues on the development of thermotolerance remains to be done.

The outcome of a combined use of heat with radiation would also be influenced by the heat-induced changes in blood flow and resultant changes in oxygen supply in tumors and normal tissues. The increases in blood flow in some types of tumors at relatively low temperatures may lead to an increase in oxygenation of tumors and, thus, an increase in the response of the tumors to subsequent radiation. Unfortunately, heat may also increase the oxygen supply in normal tissues and sensitize them to radiation. The data accumulated during the last several years suggests that an application of heat before X-irradiation exerts a more radiosensitizing effect on normal tissues than on the tumors (17, 53). It would be reasonable to attribute the above phenomenon to deterioration of blood circulation in tumors and a concomitant increase in the blood flow, and thus pO2 in normal tissues by the heat given prior to radiation.

A recent report by Hahn and Shiu (22) about the effect of pH on the combined use of hyperthermia and drugs is of interest. These investigators reported that the cytotoxic effect of some drugs, i.e., bleomycin and 1, 3-bis(2-chloroethyl)-1-nitrosourea, was markedly enhanced by heat in an acidic milieu. In this connection, Urano and Kahn (59) reported that the combination of hyperthermia, chemotherapeutic drugs, and a high dosage of glucose was more effective than were drugs plus hyperthermia in suppressing the growth of experimental mouse tumors. Perhaps the increase in intratumor acidity caused by the hyperglycemia sensitized the tumor cells to the drugs given in conjunction with hyperthermia.

It should also be pointed out that the heat-induced physiological change would inevitably influence the pharmacokinetics of drugs and thus damage the tumors and normal tissue. For example, hyperthermia at relatively high temperatures may compromize the tumor blood flow and thus reduce the amount of drug delivered to the tumors. On the other hand, heating at relatively low temperatures may increase the uptake of drugs in some tumors by virtue of an increase in blood flow. Therapeutic...
gain cannot be expected unless the changes in distribution of drugs by heat occur in favor of tumors.

In conclusion, it has been increasingly evident that the response of vascular beds to heat in tumors differs considerably from that in normal tissues. The effective clinical use of hyperthermia will depend on a careful application of these biological principles emerging from experimental work. Tumor vasculature is less able to dissipate heat and more likely to be damaged than that in normal tissues. The effective clinical use of hyperthermia and radiotherapy should optimize the different mechanisms and target populations of the 2 modalities. A rational treatment schedule must integrate 2 very different tumoricidal mechanisms: (a) the propensity of hyperthermia to exacerbate the suboptimal tumor environment; and (b) the exquisite nature of radiation to exert its effect preferentially on well-oxygenated cells. Yet, the radio sensitizing effect of heat should be maximally exploited by properly sequencing the 2 modalities. The changes in tissue oxygenation, caused by heat-induced alteration of blood flow, should be taken into account in determining the sequence of heat and radiation. Likewise, the altered drug distribution characteristics induced by blood flow changes by hyperthermia would affect the efficacy of the combined use of hyperthermia and chemotherapy. It is highly desirable to identify cytotoxic agents optimally effective in the hyperthermic tumor environment.

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References


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Chang W. Song

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