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**ORIGINAL ARTICLE**

# Implications of increased tumor blood flow and oxygenation caused by mild temperature hyperthermia in tumor treatment

C. W. SONG[1], H. J. PARK[2], C. K. LEE[1], & R. GRIFFIN[1]

[1]*Radiobiology Laboratory, Department of Therapeutic Radiology-Radiation Oncology, University of Minnesota Medical School, Minneapolis, USA and* [2]*Department of Microbiology, Medical College, Inha University, Inchon, South Korea*

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**Abstract**
In many past clinical studies in which hyperthermia enhanced the efficacy of radiotherapy, the tumor temperatures could be raised only to 40–42°C range in most cases. The heat-induced cell death, cellular radiosensitization, and vascular damage induced by such mild temperature hyperthermia (MTH) are likely to be insignificant despite the increased response of tumors to radiotherapy. Heating rodent tumors at 40–42°C was found to cause an enduring increase in blood flow and oxygenation in the tumors. Recent studies with canine soft tissue sarcoma and human tumor clinical studies also demonstrated that MTH improves tumor oxygenation, and enhances response of the tumors to radiotherapy or chemoradiotherapy. The increased blood flow and vascular permeability caused by MTH may also improve the delivery of various therapeutic agents such as chemotherapy drugs, immunotherapeutic agents and genetic constructs for gene therapy to tumor cells. MTH as a means to potentiate the efficacy of radiotherapy and others warrants further investigation.

**Keywords:** *Hyperthermia, mild temperature hyperthermia, MTH, blood flow, tumor oxygenation, tumor* $pO_2$

## Introduction

It is known that the intratumor microenvironment greatly affects the response of tumors to treatments. Five major microenvironmental factors known to influence the response of tumors to various treatments including hyperthermia are (i) Perfusion, (ii) Permeability, (iii) $pO_2$, (iv) pH and (v) Pressure (intratumor). These 5'P's greatly influence the response of tumors to heating, and in turn they are altered by heating. Among these 5'P's, perfusion or blood flow is probably most important in the hyperthermic treatment of tumors since blood flow plays the major role in heat dissipation from tumors during heating and thus heat-induced tissue damage [1–4]. Blood flow also influences the $pO_2$ and pH of tumors,

Correspondence: Chang. W. Song, Radiobiology Laboratory, Department of Therapeutic Radiology-Radiation Oncology, University of Minnesota Medical School, MMC 494, Minneapolis, MN 55455, USA. E-mail: songx001@umn.edu

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factors which greatly affect the response of tumors to radiotherapy and to certain chemotherapy drugs.

One of the original rationales for the use of hyperthermia to treat tumors was that tumor vasculature can be preferentially destroyed by heat relative to normal tissue vasculature [1–7]. Indeed, the studies conducted in our laboratory and elsewhere clearly demonstrated that the vascular beds in rodent tumors can be destroyed by heating at 42–43°C with minimal damage to normal tissue vasculature [1]. However, human tumor vasculature is more mature than animal tumor vasculature and thus heating at temperatures higher than 42–43°C may be required to cause significant damage to human tumor vessels [8, 9]. Unfortunately, heating human tumors, particularly deep-seated tumors, to temperatures sufficiently high enough to cause vascular damage using external heating devices is not an easy task. In fact, in numerous clinical studies on the efficacy of hyperthermia against various human tumors conducted during the last two decades, human tumor temperatures could be seldom raised to cytotoxic levels, i.e., >42–43°C [10–14]. These realizations strongly suggested that the increased radiosensitization in human tumors by hyperthermia at relatively mild temperatures does not result from thermal cell death and increased cellular radiosensitization but most likely from improved tumor blood flow and tumor oxygenation [10]. In this paper, we review what is known at present about the heat-induced changes in blood flow and oxygenation in animal as well as human tumors and the implications of these changes in the response of tumors to therapy, in particular, radiotherapy.

*Hyperthermia-induced changes in tumor blood flow*

Tumor vascular beds are made of thin capillary-like blood vessels, that are irregularly dilated, bulged, constricted, twisted and sharply bent [3, 4, 15]. Consequently, blood flow through such a chaotic capillary network is sluggish. Since the hastily formed tumor blood vessels mostly lack smooth muscle layer and innervation, they are unable to autoregulate. However, it should be noted that as malignant tumor tissue invades normal tissue, it incorporates normal tissue blood vessels, mainly arterioles, which are fully capable of responding to external stimuli. Therefore, the change in tumor blood flow by hyperthermia may depend, in part, on the extent of incorporation of normal arterioles in the tumor mass [15].

There have been numerous studies on the effects of hyperthermia on tumor blood flow [1–9, 16]. Following is a brief summary of the effect of hyperthermia on tumor blood flow, based mainly on work done in our own laboratory. When RIF-1 fibrosarcomas of C3H mice were heated at 44.5°C using a water bath for 1 h, blood flow began to decrease within 15 min of heating and continued to decrease to about 30% of the original value by the end of 1 h heating [17]. The tumor blood flow further decreased to undetectable levels several hours after heating at 44.5°C for 1 h. When RIF-1 tumors were heated at 43.5°C for 1 h, the blood flow increased about two-fold by the end of heating, but it rapidly decreased to negligible level thereafter. Upon heating at 41.5°C or 42.5°C for 1 h, the blood flow in the RIF-1 tumors slightly increased during heating, decreased transiently after heating, and increased within a couple hours. The blood vessels in SCK breast tumors of A/J mice were found to be more liable to heat-induced damage than RIF-1 blood flow since the blood flow in SCK tumors began to decrease after only 30 min of heating at 43.5°C and blood flow significantly decreased by the end of 1 h heating at 42.5°C [16, 18]. In a third model, R3230 adenocarcinoma of Fischer rats, heating at 40.5–43.5°C for 30 min significantly increased tumor blood flow during heating [19, 20]. When tumors were heated for 1 h at 40.5 and 41.5°C, blood flow increased and heating for 1 h at 42.5–43.5°C decreased

the tumor blood flow. An important and somewhat unexpected result in our laboratory was that in the R3230 tumors heated at 40.5 or 41.5°C for 30–60 min, the increase in tumor blood flow 24 h after heating was markedly greater than that immediately after heating. For example, the blood flow in R3230 tumors increased about 1.5 times immediately after heating while it increased 2.5 times 24 h after heating at 41.5°C for 60 min. In spontaneous canine tumors, blood flow 24 h after heating at <44°C for 1 h was found to be significantly increased while blood flow decreased after heating at T50 > 44°C [21].

### *Hyperthermia-induced changes in tumor oxygenation by mild temperature hyperthermia (MTH)*

Since oxygenation status in tumors greatly affects the efficacy of radiotherapy and also some chemotherapy drugs, the effect of hyperthermia on tumor oxygenation has received considerable attention in recent years as reviewed in recent articles [22, 23]. It is not surprising that while heating at temperatures high enough to reduce tumor blood flow decreases tumor oxygenation, heating at mild temperatures, i.e., 39–42°C, causes long-lasting increase in tumor oxygenation [22–24]. For example, the median $pO_2$ in FSaII fibrosarcomas grown in C3H mice was $6.5 \pm 0.5$ mmHg before heating and it increased to $16.6 \pm 1.1$ mmHg and $10.9 \pm 1.3$ mmHg immediately and 24 h after heating at 41.5°C for 60 min, respectively (see Figure 1) [25].

Similar heat-induced increases in $pO_2$ were observed in SCK tumors of A/J mice [26, 27]. In the R3230 tumors of rats, $pO_2$ also increased immediately and 24 h after heating at 41.5°C [19, 28]. Interestingly, in the R3230 tumors heated at 42.5°C for 60 min, the tumor $pO_2$ immediately after heating was similar to that before heating but the $pO_2$ at 24 h after heating was found to be markedly increased. The $pO_2$ in all FSaII tumors,

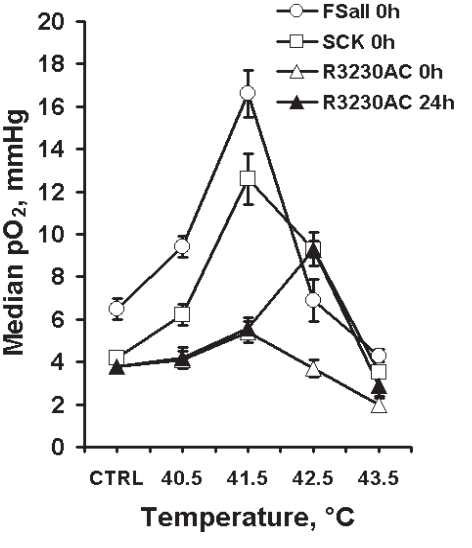


Figure 1. Heat-induced changes in $pO_2$ in FSaII fibrosarcoma of C3H mice, SCK mammary tumors of A/J mice and R3230 adenocarcinomal of Fischer rats grown s.c. in the hind legs of animals. The tumors were heated for 1 h using a water-bath at temperatures shown. The tumor $pO_2$ of FSaII tumors and SCK tumors were determined immediately after heating and that of R3230 tumors were determined either immediately or 24 h after heating. The $pO_2$ was measured using polarographic microelectrode system (Eppendorf). Each data point is the mean of more than 10 tumors. The bars show one SE of the mean. [22, 24].

SCK tumors and R3230 tumors decreased after heating at 43.5°C for 60 min. However, when R3230 tumors were heated only for 30 min, heating at 43.5°C significantly increased the $pO_2$ both immediately and 24 h after heating. Taken together, it may be concluded that, in rodent tumors, heating at mild temperature, i.e., 40–42°C, for 30–60 min increases blood flow and improves tumor oxygenation. Vujaskovic et al. [21] reported that the median $pO_2$ in canine soft tissue sarcoma was 7.9 mmHg before hyperthermic treatment and it increased to 22.6 mmHg at 24 h after the first hyperthermia treatment. Interestingly, heating the canine tumors with T50 (50% of temperature determined) lower than 44°C increased $pO_2$ in the canine tumors, while heating with T50 higher than 44°C decreased tumor $pO_2$. As we would expect, heating the tumors at T50 higher than 44°C likely caused vascular damage and thus tumors became hypoxic. In a human soft tissue sarcoma study, the average $pO_2$ before treatment was 4 mmHg and it increased about three times 24 h after a first heating at median T90 of 39.9°C (37.5–42.7°C) [11]. As much as a five-fold increase in $pO_2$ in human breast tumors 24 h after first hyperthermia treatment at a mild temperature was observed [29]. Jones et al. [14] recently reported the effect of hyperthermia on the response of locally advanced breast cancer (LABC) in humans to chemoradiotherapy. The treatment goal was to heat the tumors at 41–41.5°C for 60 min in >90% of measured points. In this study, eight of 13 tumors were hypoxic with average median $pO_2$ of 4.7 mmHg before treatment and the tumor $pO_2$ increased to 23.3 mmHg at 24 h after the first hyperthermia treatment. An important observation in this study was that heating at relatively mild temperatures was superior to heating at higher temperatures in causing reoxygenation in tumors and inducing complete tumor response to chemoradiotherapy. This result may be interpreted to mean that the high temperature heating caused vascular damage and hypoxia in the LABC tumors reducing the effectiveness of chemoradiotherapy.

### *Mechanisms of hyperthermia-induced increase in tumor oxygenation*

As mentioned before, tumor vascular beds consist of newly-formed tumor blood vessels and host normal tissue blood vessels. The host normal arterioles, which are incorporated in the tumor tissue, would dilate upon heating at mild temperatures probably due to smooth muscle relaxation via stimulation by nitric oxide synthesized by endothelial cells [22]. In this event, blood will pour into the dilated arterioles in the tumor and then subsequently flood the network of capillary-like tumor blood vessels with an increased intravascular pressure and speed. Consequently, blood may even course through previously collapsed and non-functional blood vessels, thereby reoxygenating hypoxic regions. However, it must be noted that the tissue $pO_2$ is dependent not only on oxygen supply but also on the rate of oxygen consumption by the tissue. It is likely that while the increase in tumor $pO_2$ observed during and soon after MTH results from an increase in oxygen supply through the increase in blood flow, late increases in tumor $pO_2$, i.e., 24 h after heating, is caused by both increased blood flow and decreased oxygen consumption due to some degree of heat-induced cell death or cell damage [23, 29].

### *Radiosensitization of tumors by MTH alone or in combination with carbogen breathing*

We have observed in numerous tumor models that the increase in tumor oxygenation by MTH markedly enhanced the radiosensitivity of tumor cells as determined by *in vivo-in vitro* excision assay for clonogenic cell survival and also by tumor growth delay study [24, 27, 30–32]. Figure 2 shows the results of *in vivo-in vitro* excision assay for clonogenic survival of FSaII tumor cells after various treatments [22]. In FSaII tumors, irradiation immediately

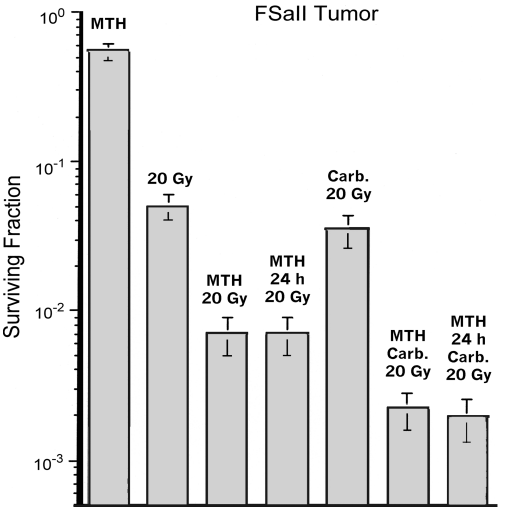


Figure 2. Surviving fraction of clonogenic cells in FSaII tumors after various treatments. MTH: Heating with 41.5°C water-bath for 1 h. 20 Gy: 20 Gy irradiation Carb: Carbogen breathing from 5 min before to end of irradiation. Tumors were irradiated either immediately or 24 h after MTH. Each treatment group represents 10 or more tumors. Mean and one SE are shown [30].

after and 24 h after MTH were equally effective in reducing cell survival. It was clear that the MTH-induced increase in tumor oxygenation sensitized the tumors to irradiation even 24 h after heating. Carbogen (mixture of 95% $O_2$ and 5% $CO_2$) breathing has been known to increase oxygen content in blood and improves tumor oxygenation [33]. However, carbogen breathing only slightly increased the radiosensitivity of FSaII tumors. In contrast, carbogen breathing markedly increased the radiation-induced cell death when tumors were pre-treated with MTH even up to 24 h before carbogen breathing and tumor irradiation. It appeared that the increase in oxygen content in blood by carbogen breathing alone is ineffective to reoxygenate the majority of hypoxic cells. The improved blood perfusion throughout tumors by prior-heating at mild temperature apparently improved the carbogen-induced reoxygenation of hypoxic cells.

## Conclusion

Mild temperature hyperthermia (MTH) causes sustained improvement of blood circulation and oxygenation in animal and human tumors. Recent clinical studies unequivocally demonstrated a link beween MTH-induced improvement of oxygenation and increases in the response of human tumors to radiotherapy or chemoradiotherapy. The radiosensitization of tumors may be further increased by combining MTH and carbogen breathing. The increase in tumor blood flow and vascular permeability caused by MTH may be exploited to increase delivery of chemotherapy drugs and other treatment agents such as drug-containing liposomes, immunotherapeutic agents and genetic constructs.

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