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Mild temperature hyperthermia and radiation therapy: role of tumor vascular thermotolerance and relevant physiological factors

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Abstract

Here we review the significance of changes in vascular thermotolerance on tumor physiology and the effects of multiple mild temperature hyperthermia (MTH) treatments on tumor oxygenation and corresponding radiation response. New information suggests that although hyperthermia is a powerful modifier of tumor blood flow and oxygenation, sequencing and frequency are central parameters in the success of MTH enhancement of radiation therapy. We hypothesize that heat treatments every 2–3 days combined with traditional or accelerated radiation fractionation may be maximally effective in exploiting the improved perfusion and oxygenation induced by typical thermal doses given in the clinic.

Keywords

mild temperature hyperthermia; vascular thermal tolerance; radiation; tumor perfusion; tumor blood flow

Introduction

The tumor microenvironment and physiology greatly affects the response of tumors to treatments, such as hyperthermia [1]. Namely, blood flow is crucial factor in the hyperthermic treatment of tumors since it controls heat dissipation from tumors during heating and subsequently heat-induced tissue damage [2]. Tumor cells die at an exponential rate when exposed to temperatures above approximately 42 °C for 30 minutes, this has been observed in pre-clinical as well as clinical settings [3,4]. Additionally, it has been shown that in animals, tumor blood flow and oxygen delivery is significantly increased when hyperthermia is applied up to 42 °C [5–8]. In human tumors, more interestingly, blood flow is even maintained and stimulated in response to temperatures as high as 45 °C [9,10]. This increase in blood flow, as well as the reduction of oxygen demand (due to hyperthermia induced cell death and metabolic suppression) in the tumor, results in significantly increased tumor tissue oxygenation [6,8,11–17]. This might make hyperthermia the best hypoxic radiosensitizer available. Here we will address the possibilities and limitations of mild temperature hyperthermia to enhance tumor radiation sensitization.

Tumor thermotolerance versus vascular thermotolerance

The phenomenon of vascular thermotolerance is a potentially powerful and clinically pertinent by-product of multiple hyperthermia treatments of the tumor [18]. The tentative definition of vascular thermotolerance can be summarized as: the blood flow response of the tumor to a second hyperthermia exposure is significantly greater than in response to a single thermal dose, even at temperatures that would normally cause vascular damage [19,20]. Namely, the delivery of two heat treatments with an interval of around 12 – 48 h between them, causes a marked increase in tumor blood flow compared to a decrease in tumor perfusion from control level when the heatings are applied consecutively with no interval [14]. Traditionally, the concept of thermotolerance *in vitro* has been a phenomenon where one heat treatment induces a temporary increase in resistance of cells against subsequent heatings. The working definition of vascular thermotolerance differs in that the measurable quantity, i.e. blood flow and oxygenation, actually increases above and beyond control level in many cases. Regardless, the general notion has been that individual cellular resistance must be at the root of the increased physiological reaction to a second heating.

In order to intelligently design clinical trials incorporating heat and radiation therapy, it is a necessity to understand the effects of single, double and even greater numbers of hyperthermia treatments. Our studies have been designed to test whether certain sequences of fractionated hyperthermia and radiation may further increase radiation sensitivity via increased oxygenation due to a 'tolerant' state of the tumor vasculature. Previous studies were focused on temperatures generally above 42–43 °C, yet current hyperthermia studies employ lower temperatures in the range of 40–42 °C since the majority of clinical hyperthermia treatments do not exceed these temperatures. Therefore, we have been focusing on 41.5 °C multi-treatment studies in line with clinically realistic temperatures and with our previous observations that this temperature maximally increase tumor oxygenation in various murine tumor models [8,14].

The influence of mild temperature hyperthermia (MTH) on tumor oxygenation

It has been shown that in both rodent as well as human tumors, varying fractions of clonogenic cells are chronically or acutely hypoxic [21–23]. The chronically hypoxic regions are allegedly caused by limited oxygen diffusion in tumor tissue due to insufficient vascular supply, and intermittent blood flow is held responsible for the acutely hypoxic regions caused by variations in interstitial pressure, clogging of vessels by immune cells or shed tumor cells and/or transient collapse of the lumens of immature tumor vessels [22,24,25].

The overall tumor oxygenation level depends on the balance of oxygen supply through blood flow and the oxygen consumption rate by tumor cells. It appears that the changes in tumor oxygenation generally parallel the changes in tumor blood flow [2,26–29]. In physiological conditions, blood flow is tightly regulated by vasodilating factors that stimulate endothelial cells to release nitric oxide, which then causes relaxation of the vascular smooth muscle and subsequent increased blood flow. We have previously demonstrated that hyperthermia increased the amount of nitric oxide synthase and overall tumoral nitric oxide production [14,29]. However, the tumor cell oxygen consumption rate has also been shown to be an important influence on tumor oxygenation levels [30]. Thermal exposure up to approximately 41 – 42 °C is able to transiently increase oxygen consumption, however above this threshold temperature the oxygen consumption rate declines [15,31]. Due to these contrasting effects of hyperthermia on the tumor oxygenation, depending on the temperature and duration applied, it is essential to delineate the different effects and

processes involved in order to rationally design the most optimal hyperthermia treatment regimens.

A single treatment of hyperthermia can have contrasting effects on tumor oxygenation; whereas mild temperature hyperthermia (MTH; i.e. 39°–41°C) improves the oxygenation levels, temperatures raised above 42°–43°C causes deterioration of the tumor oxygenation status [14,32]. Interestingly, a single mild temperature hyperthermia treatment can induce a long-lasting oxygenation improvement in murine tumors, depending on tumor type and duration of MTH [33]. Generally, multiple heatings, at various thermal dose combinations increase blood flow and oxygenation [18–20,34,35]. Figure 1 reiterates these findings and shows that in fibrosarcoma FSaII tumors [14], oxygenation remains elevated for 24–48 h post MTH at 41.5 °C for 60 min. Figure 1 also illustrates the effect of multiple daily fractionated MTH on FSaII tumor oxygenation. After one MTH treatment, the median tumor oxygenation increased from baseline 6.4 ($\pm$ 0.5) mmHg to 16.6 ($\pm$ 1.1) mmHg, which began a downward trend to 14.8 ($\pm$ 1.3) mmHg after two daily heatings, and dropped to 9.2 ($\pm$ 1.2) mmHg after three daily MTH treatments (Figure 1B).

In order to further understand the interaction of clinically relevant concomitant radiation and heating, tumor oxygenation was measured after combined treatment of MTH and radiation (Figure 1C). The median tumor oxygenation (pO₂) at 24 h after 3 Gy radiation was 5.3 ($\pm$ 1.2) mmHg, which was slightly less than the average median pO₂ in untreated FSaII tumors 6.5 ($\pm$ 0.5) mmHg. Tumors heated with MTH (41.5 °C; 60 min.) had an average median pO₂ of 10.9 $\pm$ 1.3 mmHg. When tumors were treated with MTH and immediately exposed to 3 Gy, the median pO₂ 24 h later was only 7.0 $\pm$ 2.6 mmHg. In tumors that were treated with heat and radiation, and then heated again 24 h later immediately before measuring oxygenation, the median pO₂ was found to increase to 10.0 $\pm$ 1.9 mmHg. While this was still an elevated median pO₂ as compared to untreated control tumors, radiation subdued the MTH induced tumor oxygenation enhancement, since it was significantly less than tumors treated with two treatments of MTH only (14.8 ($\pm$ 1.2) mmHg (p < 0.02)).

While it is uncertain if everyday hyperthermia is even logistically and practically feasible in the radiation oncology clinic, these studies illustrate the possibility that when combining multiple MTH and radiation treatments an interval between the hyperthermia exposures of at least 48 h may be optimal in allowing the tumor physiological response to improve radiation response.

Tumor growth response after combination of MTH and fractionated radiation

Several tumor model experimental designs were tested to elucidate whether certain sequences of fractionated hyperthermia and radiation may further increase tumor radiation sensitivity. Initially the combination of multiple consecutive MTH and radiation treatments was applied and tumor growth response was monitored in the FSaII tumor mouse model. Whereas 7 consecutive daily MTH (41.5 °C; 60 min.; schedule q1dx7) treatments inhibited tumor growth to a small extent, radiation (3 Gy for 7 days; q1dx7) caused a tumor growth delay of more than 15 days as compared to untreated control tumors (Figure 2A). The addition of MTH to each exposure of radiation caused a slight increase in tumor growth delay of approximately 2 more days, independent of whether MTH was administered prior or post radiation (Figure 2A).

In the next set of experiments intermittent MTH was tested in order to improve overall radiation response in the FSaII tumor mouse model. MTH (41.5 °C; 60 min.) was applied every other day (q2dx4) in combination with multiple radiation exposures (3 Gy for 7 days;

q1dx7). There was a noticeable increase in the tumor growth delay (5 days) when tumors were treated with MTH prior to radiation as compared to those treated post radiation (Figure 2B) which suggested that, at least qualitatively, the use of MTH before every other radiation fraction is able to improve radiation response to a greater degree than MTH after every other radiation fraction due in some part to improved tumor oxygenation. We subsequently expanded these findings in another FSaII tumor mouse model where intermittent MTH (41.5 °C; 60 min.; q2dx3) was combined with daily accelerated radiation (2 Gy, 6hrs, 2 Gy) for 5 days. Interestingly, intermittent MTH given prior to the accelerated radiation was able to enhance the radiation induced tumor growth delay (Figure 2C). The enhancement of radiation therapy response was more pronounced than when MTH was given on consecutive days (Figure 2A). Thus, a combination of multiple consecutive MTH treatments and fractionated radiation caused tumor growth delay independent of sequence while a combination of intermittent MTH and fractionated radiation caused differential tumor growth delay dependent on sequence.

One could speculate that heating every day at 41.5 °C induces sufficient accumulated vascular damage or related cellular effects over time that the enhancement in radiation response due to the increased oxygenation begins to decrease as therapy continues. Another variable that must be considered is the fact that some degree of edema is induced in tumors heating with water baths. It is conceivable that repeated edema and some degree of vascular congestion occurring every day due to repeated heat treatments could be an unintended source of vascular inefficiency and may be a factor in our results. However, our recent results (data not shown) indicate that repeated mild hyperthermia treatments cause a normalization of the murine tumor vasculature (i.e., destruction of immature and retention of mature vessels) leading to improved vascular efficiency, albeit a transient effect. Certainly, there is a great need for clinical measurements of tumor perfusion and oxygenation during the course of a thermoradiotherapy regimen involving multiple heat treatments from a non-water bath source to further develop our understanding of the tumor physiology involved. In addition, one might envision the potential inverted explanation of the concomitant radiation damage affecting hyperthermia performance. We found that the addition of 3 Gy irradiation in combination with MTH treatment suppressed the improvement in tumor oxygenation obtained by MTH alone (Figure 1C). This might be expected, since the radiation damage of both tumor parenchyma and stromal cells may reduce or inhibit normal cellular signaling that would govern the physiological reactions to hyperthermia or other treatments. While we have not investigated this exhaustively, the data presented here is a strong reminder that, clinically, tumor growth or regression is dependent on multiple factors. Therefore within a multi-modality regimen it is necessary to elucidate potential negating effects that particular treatments might inflict upon other treatment modalities. This is of particular importance since the majority of cancer patients are being treated with multi-modality regimens before, during or after thermoradiotherapy.

Regardless of the role of tumor oxygenation on the radiation response, it is also conceivable that a thermotolerant state of the tumor cells themselves could alter intrinsic radiosensitivity over the course of treatment. If true, the overall treatment response may be diminished as we observed in our study where hyperthermia and radiation were applied every day. However, most studies to date have revealed little to no direct effect of thermotolerance on the radiation responsiveness of cells, yet some reduction in thermal radiosensitization can occur [36–41]. Taken together, it appears that the induction of cellular thermotolerance has a minimal effect on the results of combined radiation and hyperthermia treatment.

MTH considerations in multi-modality treatment

Since MTH improved tumor oxygen as well as blood flow (Figure 1 and ref [14]), the delivery of various therapeutics as well as diagnostic agents could be significantly improved by proper MTH scheduling. For example, the combination of mild heating with carbogen breathing and/or nicotinamide (originally employed by themselves to improve experimental and human tumor oxygenation [23,29,42–47]) is markedly more effective in increasing tumor oxygenation and radiosensitivity than either treatment alone [29,33,48,49].

Of course, the immediate and long term effects of MTH alone on tumor perfusion and oxygenation may be part of the explanation for the positive outcomes of previous clinical trials that have combined hyperthermia and radiation [50]. In some trials, heating was applied several times per week and therefore it is conceivable that these patients' tumors acquired vascular thermotolerance, which may have maximized oxygenation and blood flow status to allow radiation and chemotherapy to exert greater cell killing in the tumor. Unfortunately, there have been little to no formal studies to date exploring the mechanisms or translational significance of this clinically relevant topic. One study assessed the changes in nanoparticle distribution after one or two hyperthermia treatments [51]. Intriguingly, this study reported that pre-heating the tumor 6–8 hours in advance, reduced the permeability and extravasation of the particles, presumably due to the development of some type of thermotolerance to the second heating [51]. Clearly, the mechanisms by which repeated hyperthermia treatments may affect chemotherapy or drug delivery are far from understood and warrant further investigation. Elucidating the mechanisms and consequences of repeated hyperthermia holds great promise for improving clinical outcomes.

To conclude, reoxygenation of previously hypoxic tumor tissue has been unmistakably shown to have important implications on radiation sensitivity [12,14,26,48]. Therefore, the induction of improved oxygenation via a single heating or via vascular thermotolerance induced by multiple heatings in human tumors may have marked effects on clinical outcomes. Our preclinical evidence suggests a threshold above which additional hyperthermia exposures may have little beneficial effect on tumor radiation response. Nonetheless, a very strong rationale for the combination of thermal therapy and cancer treatments such as hypofractionated high dose radiation therapy are supported by numerous pre-clinical studies. The potential to take advantage of substantial increases in radiation-induced cell killing in human tumors caused by thermal therapy is high, provided that we continue to characterize the tumor biology and mechanisms related to combined therapy strategies.

The data collected to date and our new observations using multiple heat and radiation exposures support the idea that two mild temperature hyperthermia (MTH; i.e. 41.5 °C; 60 min.) treatments may greatly improve tumor perfusion and oxygenation, while three or more hyperthermia exposures in a row may have a lessened influence. Moreover, that frequency and sequence are central parameters in the success of MTH enhancement of radiation therapy. These discoveries enhance our understanding of tumor thermotolerance in particular, and might challenge current multi-modality treatment dogma in general.

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References

1. Song CW, Park HJ, Lee CK, Griffin R. Implications of increased tumor blood flow and oxygenation caused by mild temperature hyperthermia in tumor treatment. *Int J Hyperthermia* 2005;21(8):761–767. [PubMed: 16338859]
2. Song CW. Effect of local hyperthermia on blood flow and microenvironment: a review. *Cancer Res* 1984;44(10 Suppl):4721s–4730s. [PubMed: 6467226]
3. Dewey WC, Thrall DE, Gillette EL. Hyperthermia and radiation--a selective thermal effect on chronically hypoxic tumor cells in vivo. *Int J Radiat Oncol Biol Phys* 1977;2(1–2):99–103. [PubMed: 849909]
4. Westermann AM, Jones EL, Schem BC, van der Steen-Banasik EM, Koper P, Mella O, et al. First results of triple-modality treatment combining radiotherapy, chemotherapy, and hyperthermia for the treatment of patients with stage IIB, III, and IVA cervical carcinoma. *Cancer* 2005;104(4):763–770. [PubMed: 15968685]
5. Song CW, Rhee JG, Haumschild DJ. Continuous and non-invasive quantification of heat-induced changes in blood flow in the skin and RIF-1 tumour of mice by laser Doppler flowmetry. *Int J Hyperthermia* 1987;3(1):71–77. [PubMed: 2951450]
6. Vujaskovic Z, Poulson JM, Gaskin AA, Thrall DE, Page RL, Charles HC, et al. Temperature-dependent changes in physiologic parameters of spontaneous canine soft tissue sarcomas after combined radiotherapy and hyperthermia treatment. *Int J Radiat Oncol Biol Phys* 2000;46(1):179–185. [PubMed: 10656391]
7. Thrall DE, Larue SM, Pruitt AF, Case B, Dewhirst MW. Changes in tumour oxygenation during fractionated hyperthermia and radiation therapy in spontaneous canine sarcomas. *Int J Hyperthermia* 2006;22(5):365–373. [PubMed: 16891239]
8. Song CW, Shakil A, Griffin RJ, Okajima K. Improvement of tumor oxygenation status by mild temperature hyperthermia alone or in combination with carbogen. *Semin Oncol* 1997;24(6):626–632. [PubMed: 9422259]
9. Acker JC, Dewhirst MW, Honore GM, Samulski TV, Tucker JA, Oleson JR. Blood perfusion measurements in human tumours: evaluation of laser Doppler methods. *Int J Hyperthermia* 1990;6(2):287–304. [PubMed: 2324570]
10. Oleson JR. Eugene Robertson Special Lecture. Hyperthermia from the clinic to the laboratory: a hypothesis. *Int J Hyperthermia* 1995;11(3):315–322. [PubMed: 7636318]
11. Griffin RJ, Okajima K, Song CW. The optimal combination of hyperthermia and carbogen breathing to increase tumor oxygenation and radiation response. *Int J Radiat Oncol Biol Phys* 1998;42(4):865–869. [PubMed: 9845112]
12. Iwata K, Shakil A, Hur WJ, Makepeace CM, Griffin RJ, Song CW. Tumour pO₂ can be increased markedly by mild hyperthermia. *Br J Cancer Suppl* 1996;27:S217–S221. [PubMed: 8763884]
13. Shakil A, Osborn JL, Song CW. Changes in oxygenation status and blood flow in a rat tumor model by mild temperature hyperthermia. *Int J Radiat Oncol Biol Phys* 1999;43(4):859–865. [PubMed: 10098442]
14. Song CW, Park H, Griffin RJ. Improvement of tumor oxygenation by mild hyperthermia. *Radiat Res* 2001;155(4):515–528. [PubMed: 11260653]
15. Thews O, Li Y, Kelleher DK, Chance B, Vaupel P. Microcirculatory function, tissue oxygenation, microregional redox status and ATP distribution in tumors upon localized infrared-A-hyperthermia at 42 degrees C. *Adv Exp Med Biol* 2003;530:237–247. [PubMed: 14562721]
16. Vaupel P, Okunieff P, Kluge M. Response of tumour red blood cell flux to hyperthermia and/or hyperglycaemia. *Int J Hyperthermia* 1989;5(2):199–210. [PubMed: 2647866]
17. Vaupel P, Kallinowski F. Physiological effects of hyperthermia. *Recent Results Cancer Res* 1987;104:71–109. [PubMed: 3296051]
18. Song CW, Lin JC, Chelstrom LM, Levitt SH. The kinetics of vascular thermotolerance in SCK tumors of A/J mice. *Int J Radiat Oncol Biol Phys* 1989;17(4):799–802. [PubMed: 2777670]
19. Lin JC, Song CW. Influence of vascular thermotolerance on the heat-induced changes in blood flow, pO₂, and cell survival in tumors. *Cancer Res* 1993;53(9):2076–2080. [PubMed: 8481910]

20. Nah BS, Choi IB, Oh WY, Osborn JL, Song CW. Vascular thermal adaptation in tumors and normal tissue in rats. *Int J Radiat Oncol Biol Phys* 1996;35(1):95–101. [PubMed: 8641932]
21. Dewhirst MW, Kimura H, Rehms SW, Braun RD, Papahadjopoulos D, Hong K, et al. Microvascular studies on the origins of perfusion-limited hypoxia. *Br J Cancer Suppl* 1996;27:S247–S251. [PubMed: 8763890]
22. Chaplin DJ, Trotter MJ, Durand RE, Olive PL, Minchinton AI. Evidence for intermittent radiobiological hypoxia in experimental tumour systems. *Biomed Biochim Acta* 1989;48(2–3):S255–S259. [PubMed: 2730619]
23. Horsman MR, Nordsmark M, Khalil AA, Hill SA, Chaplin DJ, Siemann DW, et al. Reducing acute and chronic hypoxia in tumours by combining nicotinamide with carbogen breathing. *Acta Oncol* 1994;33(4):371–376. [PubMed: 8018368]
24. Trotter MJ, Chaplin DJ, Olive PL. Effect of angiotensin II on intermittent tumour blood flow and acute hypoxia in the murine SCCVII carcinoma. *Eur J Cancer* 1991;27(7):887–893. [PubMed: 1834122]
25. Chaplin DJ, Olive PL, Durand RE. Intermittent blood flow in a murine tumor: radiobiological effects. *Cancer Res* 1987;47(2):597–601. [PubMed: 3791244]
26. Bicher HI, Hetzel FW, Sandhu TS, Frinak S, Vaupel P, O'Hara MD, et al. Effects of hyperthermia on normal and tumor microenvironment. *Radiology* 1980;137(2):523–530. [PubMed: 7433686]
27. Vaupel PW, Otte J, Manz R. Oxygenation of malignant tumors after localized microwave hyperthermia. *Radiat Environ Biophys* 1982;20(4):289–300. [PubMed: 7146320]
28. Griffin RJ, Okajima K, Ogawa A, Song CW. Radiosensitization of two murine tumours with mild temperature hyperthermia and carbogen breathing. *Int J Radiat Biol* 1999;75(10):1299–1306. [PubMed: 10549607]
29. Griffin RJ, Ogawa A, Williams BW, Song CW. Hyperthermic enhancement of tumor radiosensitization strategies. *Immunol Invest* 2005;34(3):343–359. [PubMed: 16136785]
30. Kirkpatrick JP, Brizel DM, Dewhirst MW. A mathematical model of tumor oxygen and glucose mass transport and metabolism with complex reaction kinetics. *Radiat Res* 2003;159(3):336–344. [PubMed: 12600236]
31. Vaupel P, Ostheimer K, Muller-Klieser W. Circulatory and metabolic responses of malignant tumors during localized hyperthermia. *J Cancer Res Clin Oncol* 1980;98(1):15–29. [PubMed: 7451551]
32. Vujaskovic Z, Song CW. Physiological mechanisms underlying heat-induced radiosensitization. *Int J Hyperthermia* 2004;20(2):163–174. [PubMed: 15195511]
33. Okajima K, Griffin RJ, Iwata K, Shakil A, Song CW. Tumor oxygenation after mild-temperature hyperthermia in combination with carbogen breathing: dependence on heat dose and tumor type. *Radiat Res* 1998;149(3):294–299. [PubMed: 9496893]
34. Rhee JG, Song CW, Levitt SH. Changes in thermosensitivity of mouse mammary carcinoma following hyperthermia in vivo. *Cancer Res* 1982;42(11):4485–4489. [PubMed: 7127290]
35. Song CW, Patten MS, Chelstrom LM, Rhee JG, Levitt SH. Effect of multiple heatings on the blood flow in RIF-1 tumours, skin and muscle of C3H mice. *Int J Hyperthermia* 1987;3(6):535–545. [PubMed: 3693986]
36. Campbell SD, Kruuv J, Lepock JR. Characterization and radiation response of a heat-resistant variant of V79 cells. *Radiat Res* 1983;93(1):51–61. [PubMed: 6823509]
37. Dikomey E, Jung H. Effect of thermotolerance and step-down heating on thermal radiosensitization in CHO cells. *Int J Radiat Biol* 1992;61(2):235–242. [PubMed: 1351911]
38. Fortin A, Raybaud-Diogene H, Tetu B, Deschenes R, Huot J, Landry J. Overexpression of the 27 KDa heat shock protein is associated with thermoresistance and chemoresistance but not with radioresistance. *Int J Radiat Oncol Biol Phys* 2000;46(5):1259–1266. [PubMed: 10725639]
39. Hartson-Eaton M, Malcolm AW, Hahn GM. Radiosensitivity and thermosensitization of thermotolerant Chinese hamster cells and RIF-1 tumors. *Radiat Res* 1984;99(1):175–184. [PubMed: 6739722]
40. Mackey MA, Anolik SL, Roti Roti JL. Changes in heat and radiation sensitivity during long duration, moderate hyperthermia in HeLa S3 cells. *Int J Radiat Oncol Biol Phys* 1992;24(3):543–550. [PubMed: 1399742]

41. Mivechi NF, Li GC. Lack of effect of thermotolerance on radiation response and thermal radiosensitization of murine bone marrow progenitors. *Cancer Res* 1987;47(6):1538–1541. [PubMed: 3815353]
42. Chaplin DJ, Horsman MR, Aoki DS. Nicotinamide, Fluosol DA and Carbogen: a strategy to reoxygenate acutely and chronically hypoxic cells in vivo. *Br J Cancer* 1991;63(1):109–113. [PubMed: 1846549]
43. Laurence VM, Ward R, Dennis IF, Bleehen NM. Carbogen breathing with nicotinamide improves the oxygen status of tumours in patients. *Br J Cancer* 1995;72(1):198–205. [PubMed: 7599052]
44. Rojas A, Joiner MC, Denekamp J. Extrapolations from laboratory and preclinical studies for the use of carbogen and nicotinamide in radiotherapy. *Radiother Oncol* 1992;24(2):123–124. [PubMed: 1386678]
45. Brizel DM, Lin S, Johnson JL, Brooks J, Dewhirst MW, Piantadosi CA. The mechanisms by which hyperbaric oxygen and carbogen improve tumour oxygenation. *Br J Cancer* 1995;72(5):1120–1124. [PubMed: 7577456]
46. Noth U, Rodrigues LM, Robinson SP, Jork A, Zimmermann U, Newell B, et al. In vivo determination of tumor oxygenation during growth and in response to carbogen breathing using ¹⁵C5-loaded alginate capsules as fluorine-19 magnetic resonance imaging oxygen sensors. *Int J Radiat Oncol Biol Phys* 2004;60(3):909–919. [PubMed: 15465209]
47. Robinson SP, Howe FA, Stubbs M, Griffiths JR. Effects of nicotinamide and carbogen on tumour oxygenation, blood flow, energetics and blood glucose levels. *Br J Cancer* 2000;82(12):2007–2014. [PubMed: 10864210]
48. Griffin RJ, Okajima K, Barrios B, Song CW. Mild temperature hyperthermia combined with carbogen breathing increases tumor partial pressure of oxygen (pO₂) and radiosensitivity. *Cancer Res* 1996;56(24):5590–5593. [PubMed: 8971160]
49. Ogawa A, Griffin RJ, Song CW. Effect of a combination of mild-temperature hyperthermia and nicotinamide on the radiation response of experimental tumors. *Radiat Res* 2000;153(3):327–331. [PubMed: 10669555]
50. Dewhirst MW, Prosnitz L, Thrall D, Prescott D, Clegg S, Charles C, et al. Hyperthermic treatment of malignant diseases: current status and a view toward the future. *Semin Oncol* 1997;24(6):616–625. [PubMed: 9422258]
51. Kong G, Braun RD, Dewhirst MW. Characterization of the effect of hyperthermia on nanoparticle extravasation from tumor vasculature. *Cancer Res* 2001;61(7):3027–3032. [PubMed: 11306483]
52. Griffin RJ, Lee SH, Rood KL, Stewart MJ, Lyons JC, Lew YS, et al. Use of arsenic trioxide as an antivasculature and thermosensitizing agent in solid tumors. *Neoplasia* 2000;2(6):555–560. [PubMed: 11228548]
53. Kallinowski F, Zander R, Hoeckel M, Vaupel P. Tumor tissue oxygenation as evaluated by computerized-pO₂-histography. *Int J Radiat Oncol Biol Phys* 1990;19(4):953–961. [PubMed: 2211264]
54. Vaupel P, Schlenger K, Knoop C, Hockel M. Oxygenation of human tumors: evaluation of tissue oxygen distribution in breast cancers by computerized O₂ tension measurements. *Cancer Res* 1991;51(12):3316–3322. [PubMed: 2040005]
55. Dings RP, Williams BW, Song CW, Griffioen AW, Mayo KH, Griffin RJ. Anginex synergizes with radiation therapy to inhibit tumor growth by radiosensitizing endothelial cells. *Int J Cancer* 2005;115(2):312–319. [PubMed: 15688384]
56. Dings RP, Yokoyama Y, Ramakrishnan S, Griffioen AW, Mayo KH. The designed angiostatic peptide anginex synergistically improves chemotherapy and antiangiogenesis therapy with angiostatin. *Cancer Res* 2003;63(2):382–385. [PubMed: 12543791]

Figure 1.

Tumor oxygenation as function of MTH and radiation. **(A)** A single application of MTH (41.5 °C; 30 or 60 min.) in FSaII murine fibrosarcoma and SCK murine breast carcinoma induces a lasting elevation of median oxygenation up to 48 hours [14]. **(B)** The extent of reoxygenation diminishes as a function of repeated daily MTH (41.5 °C; 60 min.) in FSaII tumors. Oxygenation was measured immediately after the treatments. **(C)** Radiation reduces MTH induced increased tumor oxygenation. Radiation (3 Gy) reduces the MTH (41.5 °C; 60 min.) induced increased tumor oxygenation measured 24 hrs after the treatments (middle bars), as well as when radiation (3 Gy) is combined with 2 daily MTH (41.5 °C; 60 min.) treatments and measured immediately after the treatment (bars on the right).

The pO₂ values of all panels is reported are the average of 120 to 700 individual readings derived from 9 – 15 mice per group per day, with 4 tracks per mouse and 10 values per track. * $p < 0.02$. Student's t test. Tumors were heated by immersing the tumor-bearing legs of anesthetized mice into preheated water for 60 min, as described previously [52]. The tumor pO₂ was measured with an Eppendorf pO₂ Histogram (Eppendorf, Hamburg, Germany). Tumor-bearing animals were laid on a Plexiglas board and the tumor-bearing legs were gently stretched and affixed by taping the foot to the board. A pO₂ electrode (300 μ m diameter, Eppendorf, Hamburg, Germany) was inserted by hand into the tumors through small incisions made in the skin over the distal side of the tumor. The electrode was then advanced by a computer-controlled system measuring pO₂ along the track: the electrode was advanced by 0.7 mm forward steps, immediately withdrawn by 0.3 mm to reduce the compression pressure and the pO₂ value was recorded [48,53,54]. Tumors were locally irradiated with a Philips 250 Kv X-ray machine at a dose rate of 1.4 Gy/min. The body was shielded with lead and only the tumor and foot exposed to the X-ray beam, as described earlier [55].

Figure 2.

The effect of MTH on FSaII tumor growth delay by radiation. **(A)** Daily exposures of radiation (3 Gy) and MTH (41.5 °C; 60 min.) for 7 consecutive days (schedule q1dx7) increased FSaII tumor growth delay to a modest extent compared to radiation alone. -■- control; -●- MTH (41.5 °C; 60 min.; q1dx7); -▼- radiation (3 Gy; q1dx7); -◄- radiation prior to MTH; -►- MTH prior to radiation. Data points represent the means (n = 10 animals per group) ± SEM. Arrows indicate treatment schedule. **(B)** MTH (41.5 °C; 60 min.) applied every other day prior to daily irradiation (3 Gy) increased FSaII tumor growth delay as compared to MTH given post radiation. -■- control; -●- MTH (41.5 °C; 60 min.; q2dx4); -◄- radiation (3 Gy; q1dx7) prior to MTH; -►- MTH prior to radiation. Data points

represent the means ($n = 9 - 10$ animals per group) $\pm$ SEM. (C) MTH applied every other day before accelerated radiation therapy significantly improves tumor response to radiotherapy. -■- control; -●- MTH (41.5 °C; 60 min.; q2dx3); -▼- radiation (daily 2×2 Gy with 6hr interval); -►- MTH prior to radiation. Data points represent the means ($n = 8 - 10$ animals per group) $\pm$ SEM. Arrows alone indicate radiation schedule; arrows with box shape addition indicate MTH and radiation treatment. The FSaII tumors were grown and handled as described earlier [14], with the slight modification that the mice were randomized and treatments were initiated when tumors reached at least an average of 200 mm³ in size. Tumor volume was measured with a caliper (Scienceware) and calculated according the equation: $(a^2 \times b) / 2$, where 'a' is the width and 'b' the length of the tumor [56]. Tumors were heated by immersing the tumor-bearing legs of anesthetized mice into preheated water for 60 min, as described previously [52]. Tumors were locally irradiated with a Philips 250 Kv X-ray machine at a dose rate of 1.4 Gy/min. The body was shielded with lead and only the tumor and foot exposed to the X-ray beam, as described earlier [55].