Hyperbaric Oxygen: Does it promote growth or recurrence of malignancy?

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Feldmeier J, Carl U, Hartmann K, Sminia P, Hyperbaric oxygen: Does it promote growth or recurrence of malignancy? Undersea Hyperb Med; 30(1): 1-18 - It has been a concern that a therapeutic modality recommended as an adjunct to healing and administered to promote proliferation of fibroblasts, epithelial cells and blood vessels in a wound could also lead to proliferation of malignant cells and angiogenesis in a malignant tumor. The first reported concern that hyperbaric oxygen (HBO_2) might have cancer growth enhancing effects appeared in a paper by Johnson and Lauchlan in 1966. In a series of patients treated with HBO_2 radiosensitization, they reported a more frequent than expected incidence of metastases and an unusual pattern of metastases. The published literature from clinical reports, animal studies and cell culture studies are reviewed. Putative mechanisms whereby HBO_2 could have carcinogenic effects are discussed. The processes of angiogenesis in wound healing and in cancer growth are compared and contrasted. *In vitro, in vivo* and clinical studies strongly suggest no more than a neutral effect of HBO_2 on tumor growth. In fact some studies suggest a negative impact of HBO_2 on malignant progression or formation. For angiogenesis, similarities in wound healing and cancer are striking but significant differences are found including the relative importance of angiogenic factors and the process of cessation of angiogenesis. Tumors that grow in hypoxic environments are more prone to metastases and more lethal to the patient. They are also more likely to mutate toward resistant genotypes. Discussion of postulated mechanisms of carcinogenesis including free radical and immunosuppressive effects points out why they are not likely to enhance or cause cancer growth or initiation. In conclusion, the published literature on tumor angiogenesis mechanisms and other possible mechanisms of cancer causation or accelerated growth provides little basis for HBO_2 to enhance malignant growth or metastases. A history of malignancy should not be considered a contraindication for HBO_2 therapy.

Hyperbaric oxygen, carcinogenesis, metastasis, angiogenesis, free radicals, immune suppression

INTRODUCTION

Practitioners of hyperbaric medicine have had concerns that a therapeutic modality which is recommended as an adjunct to healing and administered to promote proliferation of fibroblasts, epithelial cells and blood vessels in a wound could also lead to proliferation of cancer cells and angiogenesis in a malignant tumor. Since cellular and vascular proliferation is promoted by HBO_2 in a healing wound, we might assume that it would have the same effect in a tumor. This line of reasoning is indeed understandable at first glance, but it fails to recognize important differences between the complex physiology of wound healing and the equally complex and unique pathophysiology of malignant transformation, tumor growth and metastases.

The first reported concern that hyperbaric oxygen might have cancer growth enhancing effects appeared in a paper by Johnson and Lauchlan in 1966.[1] These authors published their experiences in irradiating 25 patients with Stage III or IV Cervical Cancer utilizing HBO_2 as a radiosensitizer at the time of irradiation. They reported a more frequent than expected incidence of metastases and a pattern of metastases that appeared to be unusual. This publication was followed by a number of larger human trials, all from the experience in applying HBO_2 as a radiosensitizer. Additionally, a number of animal trials followed specifically designed to address HBO_2's effect on primary tumor and metastatic growth. Several *in vitro* studies also have been published that address these concerns. This issue is still of concern to some.[2] This review is an update of work presented at a Consensus Conference in Lisbon, Portugal in 2001 jointly sponsored by ESTRO (The European Society of Therapeutic Radiation and Oncology) and the ECHM (The European Committee for Hyperbaric Medicine) [3] and convened to critically evaluate the efficacy of HBO_2 for a wide range of radiation injuries. The conference organizers felt that a review of the available information related to HBO_2 as a possible promoter of malignant growth was necessary to evaluate this topic using a risk-benefit approach.

The discussion of this important issue will begin with a review of the published pre-clinical studies (*in vitro* and animal) followed by clinical publications. We will also discuss putative mechanisms whereby HBO_2 could potentially have malignant growth promoting effects. Possible mechanisms of carcinogenesis and malignant growth enhancement will include discussion of a direct effect on cancer growth, immune suppression, free radical formation and mutagenesis. Most of this discussion will be incorporated into the section on pre-clinical experience since pre-clinical studies seek to investigate basic biochemical and pathophysiologic mechanisms. Additional discussion will emphasize tumor angiogenesis mechanisms since recent concerns have related to HBO_2's effect as an inducer of angiogenesis. We will also deal broadly and simultaneously with the concerns of enhanced carcinogenesis and enhanced metastatic growth but recognize the important differences in the pathophysiology of each entity.

PRE-CLINICAL STUDIES

The effect of HBO_2 on tumor cells in cell culture and tumor growth in animal models has been studied often. In terms of a potential impact on initiating or enhancing malignant growth, the reports can be divided into the following categories: 1. Direct effects of HBO_2 on cell growth in culture; 2. Effects of HBO_2 on immune competency; 3. Effects of HBO_2 on free radical formation; 4. Effects of HBO_2 on mutagenesis (generally as a result of free radical formation); and 5. Animal models of tumor growth and metastases.

Direct Effects on Cells in Cell Culture

Kalns[4] and associates have reported the effects of HBO_2 on the growth of two prostate cancer cell lines in cell culture. In this study, both cell lines had their growth suppressed after exposure to 100% oxygen for 90 minutes at 3.0 ATA relative to normobaric controls by 8.1% and 2.7% respectively.

Feldmeier[5] and associates in abstract form have reported a dose dependent reduction of numbers of colonies of B16 (amelanotic melanoma) cells grown in cell culture by increasing HBO_2 pressure or exposure time. In this study, cells exposed to HBO_2 were also less likely to adhere to fibronectin substrata in the culture media suggesting decreased metastatic potential.

The ability of cells to adhere to vascular endothelium prior to migration through the epithelium is a prerequisite for successful metastasis.

The studies cited above demonstrate an inhibitory direct effect on tumor cell growth in cell culture and suggest an effect, which may decrease metastatic potential. Caution should be used in interpreting both of these reports because the oxygen tensions experienced by the cells in culture are much higher than those cancer cells would experience *in vivo* in either an animal model or in human patients.

Hyperbaric Effects on Immune Competence

Cancer incidence and progression are known to increase during chronic immune suppression including organ transplant patients and patients with HIV. A number of publications report immune suppression by HBO_2 mostly in animal models and as a result of extreme pressure and time exposures. In 1997, Xu[6] and colleagues have shown in rodents a decrease of certain lymphocyte subpopulations in the spleen and thymus after HBO_2, but no delay in T cell response to Con A was observed. Brenner and associates[7] recently reported depression of several immune parameters including weakening of response to antigens, slowing of allograft rejection and weakening of autoimmune response after hyperbaric exposures. They suggest that such effects are offset by acclimatization. Feldmeier and associates[8] found no effects on a broad range of immune parameters in healthy human volunteers exposed to a typical clinical course of HBO_2.

The above studies do not consistently demonstrate a frequency or degree of immune suppression likely to promote malignant growth. The study by Brenner suggests that adaptation does occur in human subjects. Even if prolonged extreme hyperbaric exposures are immune suppressive, the intermittent nature of therapeutic HBO_2 allows for recovery. Typically a patient receives 90 to 120 minutes of HBO_2 daily. It is likely that the other 22 to 22 and 1/2 hours permit normalization of immune competency. This is suggested by the reports of Brenner[7] and Feldmeier[8] above.

Lash and co-workers[9] have reported that *hypoxia* not hyperoxia leads to decreased immune response by NK (natural killer cells) when tumor cells in cell culture are grown in 1% vs. 20% oxygen.

Hyperbaric Oxygen Effects on Free Radical Damage

Free radicals contribute to the development of a number of diseases including cancer. . Several recent studies suggest that exposure to HBO_2 does not necessarily cause increased free radical damage. Kaelin and associates[10] have shown a significant increase in the activity of the anti-oxidant enzyme superoxide dismutase in animals exposed to HBO_2. Zamboni and his collaborators[11] failed to demonstrate signs of increased free radical damage by hyperbaric exposure in an animal model of reperfusion injury. On the other hand, Monstrey et al[12] showed an increase in soft tissue damage in a model of Adriamycin extravasation in animals exposed to HBO_2 both before and after the extravasation. The authors attribute this additional damage to increased free radical activity although other mechanisms are not considered. Elayan and associates[13] showed no evidence of increased levels of 2,3-dihydroxybenzoic acid (a marker of hydroxyl radical generation) in Sprague-Dawley rats exposed to HBO_2 at 3.0 ATA.

The available scientific information does not conclusively demonstrate an increase in free radical damage induced by HBO_2. Again the intermittent nature of the hyperbaric exposure probably reduces the effects of any increase in reactive oxygen species. Adaptive mechanisms,

which lead to an increase in free radical scavenging, also seem to reduce the deleterious effects of any additional free radical generation.

Mutagenesis and Subsequent Carcinogenesis

Several authors have voiced concerns about mutagenesis and resultant carcinogenesis caused by free radical generation as a result of HBO_2 exposure. In 1985, Ceruti[14] proposed that HBO_2 could be carcinogenic as are some other pro-oxidants. This paper presents no evidence of a carcinogenic or mutagenic effect of HBO_2 but instead discusses known effects of oxygen and reactive oxygen species and assumes HBO_2 will necessarily increase free radical damage including mutation and carcinogenesis. Interestingly, several of the author's key references were not reports of true hyperbaric exposure but instead of prolonged exposure to increased concentrations of normobaric oxygen.[15,16] The author extrapolates these results and assumes that such observations would be augmented at hyperbaric pressures. Similar reasoning had suggested that HBO_2 was contraindicated in ischemia-reperfusion injuries since it was assumed that exposure to HBO_2 under these circumstances would increase free radical generation and resultant damage. Investigators demonstrating beneficial effects of HBO_2 in iscemia-reperfusion injury including the induction of free radical scavengers as already discussed above dispute this rationale. A group from the University of Ulm have studied the effects of HBO_2 on mutations in leukocytes of healthy human volunteers exposed to 2.5 ATA.[17] In this study and follow on studies, changes were seen in levels of 8-OHguanine (a major DNA modification induced by reactive oxygen species).[17,18] Also no mutation induction at the HPRT locus was detected. This too is a standard test for mutagenesis. DNA damage was demonstrated by the comet assay and mutations were demonstrated in the mouse lymphoma assay (MLA). The authors suggest that mutations observed due to hyperbaric exposures are clastogenic, i.e. the result of DNA strand breaks.

Speit and colleagues[19] have investigated the effects of HBO_2 on human blood cells making use of both *in vivo* and *in vitro* assays. These do show oxidative genotoxicity; however, the authors do admit that even though there was genotoxicity in their model, cancer-inducing effects have not been demonstrated for HBO_2.

There is no doubt that reactive oxygen species can under some circumstances cause mutations that may lead to carcinogenesis. Available information does not provide consistent evidence of hyperbaric induced mutagenesis. Some *in vitro* studies do show mutagenesis in cells in cell culture. Again we should observe the caution that oxygen levels achievable in cell cultures are much higher than those achievable *in vivo.* Furthermore, *in vitro* studies and *in vivo* studies, which only involve a single exposure or a short course of exposure, may not allow for the development of protective mechanisms such as the induction of free radical scavengers. The intermittent rather than continuous exposure of patients to HBO_2 likely permits repair of many DNA breaks that may result during clinical treatments. Bruyninckx and associates[20] pointed out in 1978 that oxygen levels that are mutagenic in sensitive cells in cell culture may be physiologic in humans. This observation brings into question the relevance of cell culture studies that show mutagenesis due to HBO_2 exposure in terms of carcinogenesis in humans.

Animal Studies of Tumor Growth and Metastasis

In response to Johnson and Lauclan's[1] publication, a number of researchers set out to investigate the effects of HBO_2 on animals with transplanted, induced or spontaneous tumors. Table 1 lists 17 animal studies specifically designed to address the issue of whether HBO_2

exposure of animals led to enhanced growth of the dominant tumor mass or resultant metastases. The first study was published in 1966 and the last in 2001.[21-37] Fifteen of the 17 studies show no increase in primary or metastatic growth. Two studies that show evidence of enhanced growth are mixed in their results. The paper by Shewell and Thompson[29] shows an increase in the rate of lung metastases for spontaneous mammary tumors in mice while in the same study transplanted tumors had identical rates of growth and metastases in control and hyperbaric groups. The increase in incidence of lung metastases in the spontaneous tumor group does not achieve statistical significance. In the paper by McMillan et al[32] with an anthracene induced tumor in a hamster cheek pouch model, animals exposed to HBO_2 had fewer but larger tumors. In an almost identical model, Marx and Johnson[30] showed a delay in the development of cancers in animals exposed to HBO_2. Six of the studies in Table 1 actually show some evidence of decreased tumor growth or metastases in animals exposed to HBO_2. Mostly this decrease is seen as a trend and not in a statistically or clinically significant fashion. Please note that the Table identifies studies with enhancing, inhibitory, neutral or mixed results.

Taken together these animal studies demonstrate no worse than a neutral effect by hyperbaric oxygen on the growth of induced, transplanted and spontaneous tumors and their secondary metastases. It is important to note that a broad range of tumor types and histologies were investigated in these studies. The tumors studied include squamous cell carcinomas, adenocarcinomas (mammary tumors), melanomas, leukemias and sarcomas. Some have suggested that HBO_2 may stimulate growth in one tumor and not another. The consistent results in a broad spectrum of tumor types fail to support this proposition.

HUMAN STUDIES

Fifteen clinical reports are given in Table 2.[1,38-51] These list the publications from which we can analyze the effects of hyperbaric oxygen on recurrence or metastases in patients exposed to HBO_2. Twelve of the 15 publications come from studies published to report the efficacy of HBO_2 as a radio-sensitizer. The study by Van Den Brenk et al[38] compared outcome in a group of head and neck cancer patients radiosensitized by HBO_2 and compared outcome to a historic control group. Also the study by Denham[49] and associates compared patients irradiated under hyperbaric conditions to historic controls. Likewise, the original publication by Johnson and Lauchan[1] suggesting increased metastases was not a controlled trial. The remainder of the radio-sensitization studies were randomized and controlled. These studies were not specifically designed to address the issue of the effect of hyperbaric oxygen on primary growth or metastasis. The focus of our review in table 2 centers on incidence of metastases and survival of the patients since the control or growth of the primary tumor was impacted by the radiation, which the patient received as the primary endpoint of these studies. Destruction of the primary tumor was consistently improved in the hyperbaric group compared to the air controls. Often, this improvement in local control did not translate into a survival advantage for the patients.

Ten of these twelve studies are clearly neutral or advantageous in terms of patient survival or incidence of metastases. The original paper by Johnson and Lauchan [1] that first voiced concerns of enhanced tumor growth under hyperbaric conditions is refuted by a larger experience in cervical cancer by the same author. [40] The report by Cade [39] and associates is a mixed study wherein the hyperbaric group radio-sensitized for lung cancer had no increased metastases; whereas the bladder cancer patients receiving hyperbaric oxygen had increased

Table 1: Animal Studies

Author	YEAR	Details of Report
McCredie, et al[21] ↔	1966	C3HBA mouse mammary tumor; no effect on primary or metastasis.
Suit, et al[22] ↔	1966	Strong A and BDF mouse mammary tumor; no effect on primary or metastasis.
DeCosse, et al[23] ↓	1966	For mouse melanoma decrease in pulmonary metastases; no change in primary growth.
Johnson, et al[24] ↔	1967	Mouse melanoma and leukemia. For melanoma no increase in primary or size or number of metastases For leukemia no decrease in survival.
Dettmer, et al[25] ↓	1968	Rat carcinosarcoma; both primary and metastases decreased in HBO_2 arm.
Evans, et al[26] ↔	1968	Mouse skin cancer; same incidence of lung metastases.
Feder, et al[27] ↔	1968	Implanted rhabdomyosarcoma in mice; metastases identical in HBO_2 group.
Johnson, et al[28] ↔	1971	Transplanted lymphoblastic leukemia; no difference in survival, primary tumor growth or metastases.
Shewell et al[29] ↑ ↔	1980	Two separate studies: Both transplanted and spontaneous murine mammary tumors; for spontaneous tumors 88.8% mets in HBO_2 vs 66.6% in air; otherwise primary tumor and mets in transplanted tumor identical.
Marx, et al[30] ↓	1988	DMBA induced SCCA in hamsters; delayed growth in HBO_2 Group
Frid et al[31] ↔	1989	No increase in growth of transplanted tumor or metastases in transplanted sarcoma and melanoma in murine model
McMillan, et al[32] ↑ ↓	1989	DMBA induced tumors in hamsters; larger but fewer tumors in HBO_2 vs air .
Mestrovic, et al [33] ↓ ↔	1990	Suppression of metastatic tumors in lung after IV injection of anaplastic tumor; no change in growth when transplanted in hind limb
Headley, et al [34] ↔	1991	Human SCCA xenografts in nude mice; no difference in growth
Sklizovic, et al[35] ↔	1993	Human xenotransplants of SCCA in mice; HBO_2 group received 21 treatments; No difference in tumor weight, volume or histology compared to control
Lyden, et al[36] ↔	1997	MCG 101 Sarcoma transplanted in mice; HBO_2 exposed to 2.8 ATA for 9 days; compared to control in HBO_2 group accumulation of cells in S-phase but no change in tumor growth
Takiguchi et al [37] ↓	2001	In sarcomas transplanted into mice growth slightly inhibited by exposure to HBO_2

↑ indicates increased growth; ↓ indicates decreased growth; ↔ indicates no effect on growth. If 2 symbols are given, the effect is mixed.

metastases. The bladder cancer patients in the control and hyperbaric groups were not well matched. There were increased numbers of patients in the hyperbaric group with advanced stage and more aggressive histologies. Outcome of treatment for patients with bladder cancer is substantially worse for advanced and poorly differentiated tumors. Most of the trials of hyperbaric radio-sensitization involve patients with squamous cell cancers of the head and neck or cervix. This type of patient was favored for enrollment into these trials because local control in this group is often tantamount to cure. Tumors of the head and neck and cervix typically do not metastasize until quite late in their course.

The other 3 studies present anecdotal experiences in patients with a history of malignancy who undergo a course of HBO_2 as treatment for radiation injury or non-healing wounds. One is a report of 3 patients with paralysis secondary to spinal cord injury who had had HBO_2 for pressure ulcers and were found to have urothelial tumors which progressed rapidly after discovery.[45] Two of the 3 patients had indwelling catheters for many years. The authors discuss long-term usage of catheters for bladder drainage as a risk factor for urothelial tumors. The authors also report that another 113 patients with spinal cord injury were given HBO_2 at their facility for various reasons and that none of these patients developed malignancy. Bradfield and associates[47] in 1996 reported 4 head and neck patients with advanced head and neck cancer who were treated with HBO_2 for radiation injury and had recurrence and rapid progression of their malignancies thereafter. All 4 patients had originally presented with advanced cancers. Two had already had recurrence before HBO_2. Another patient had his irradiation delayed by 6 months after surgery as a result of pneumonia. Delayed initiation of radiation as an adjunct to surgery is well known to increase the likelihood of recurrence. All together this group represented a group in whom tumor progression was no surprise.

Finally, Marx[48] reviewed his experience in 245 patients who received HBO_2 for radiation injury. He compares this to another group of 160 patients treated by him for radiation injury but who did not receive hyperbaric treatments. The hyperbaric group had a recurrence rate of 19.6% compared to 28% in the non-hyperbaric group.

If we sum the results from Table 2, we find that a total of 72 patients were involved in studies with recurrent or progressive cancer (including those in the control groups). The studies reporting a neutral or inhibitory effect for hyperbaric oxygen included over 3,000 patients The force of this clinical experience fails to support concerns that HBO_2 enhances malignant growth.

ANGIOGENESIS

Introduction

The coordinated steps needed for angiogenesis in wound healing and tumor growth are very complex and far from completely understood. Recent medical discoveries begin to shed some light on these very involved processes. This discussion is meant to present a brief synopsis of the presently understood mechanisms and to consider the effects of hyperbaric oxygen on tumor angiogenesis based on what we know and what we can postulate based on indirect evidence. Before we begin, consider that angiogenesis is not only important in tumor growth and wound healing but also in myocardial ischemia and diabetic retinopathy. There is no ground swell of concern that HBO_2 pathologically increases angiogenesis in diabetic retinopathy and no evidence to suggest that therapeutic HBO_2 enhances angiogenesis in coronary artery disease. We suggest from the outset that there are definite similarities between wound healing and cancer angiogenesis but we should be cognizant of the important differences as well.

Table 2: Clinical Reports

AUTHOR	YEAR	Details of Report
Johnson, et al[1] ↑	1966	25 patients HBO_2 radiosensitized for cervical cancer showed unusual frequency and pattern of metastases; 30 exposures at 3.0 ATA
Van DenBrenk, et al[38] ↓	1967	85 head and neck patients with historic controls; had statistically significant decrease in metastases in HBO_2 Group; 2-6 exposures at 3.0 ATA
Cade, et al[39] ↔ ↑	1967	Controlled trial of 49 patients with lung CA and 40 patients with bladder CA; Metastases the same in HBO and control for lung but increased in bladder HBO_2 group; not well matched for tumor grade; 40 exposures at 3.0 ATA
Johnson, et al[40] ↔ ↓	1974	Controlled trial of 64 cervical cancer patients; metastases identical in HBO_2 and control groups; 5 yr survival 44% HBO_2 vs 16% control; 25-30 exposures at 3.0 ATA
Henk, et al[41] ↔ ↓	1977	Controlled trial of 276 head and neck cancers; rates of metastasis identical for HBO_2 and air groups; recurrence-free survival better in HBO group; 10 exposures at 3.0 ATA
Henk, et al[42] ↓	1977	Controlled trial of 104 head and neck cancers; disease-free survival statistically improved in HBO_2 patients; 10 exposures at 3.0 ATA
Bennett, et al[43] ↔	1977	Controlled trial of 213 cervical cancers; no increased metastases in HBO_2 group; 10 exposures at 3.0 ATA
Perrins, et al[44] ↔	1978	Controlled trial of 236 bladder cancers; no difference in survival at 4 yrs and no difference in metastases; 6-40 exposures at 3.0 ATA
Watson, et al[45] ↔	1978	Controlled trial of 320 cervical cancers; metastases identical in HBO_2 and control groups; 6-27 exposures at 3.0 ATA
Dische et al[46] ↔	1978	Controlled trial of 1500 patients with head and neck, bladder, bronchus or cervical cancer; No difference in metastases from HBO_2 to control; 6-12 exposures at 3.0 ATA
Brady, et al[47] ↓	1981	Controlled trial of 65 cervical cancers; distant failure higher in control (34%) vs HBO_2 group (16%); 10 exposures at 3.0 ATA
Eltorai, et al[48] ↑	1987	3 anecdotal cases of urothelial cancer patients in patients with chronic spinal cord injury whose cancer progressed rapidly after HBO_2; 10-20 exposures at 2.0 ATA
Denham, et al[49] ↓	1987	201 patients irradiated for head and neck cancer with hyperbaric radio-sensitization; tumor control and survival better than historic controls
Bradfield, et al[50] ↑	1996	The authors present 4 cases of head and neck cancer which rapidly progressed after HBO_2 exposure; all were advanced; 2 had prior recurrences; 1 had radiation interrupted for 6 wks because of pneumonia
Marx[51] ↓	1999	The author presents 405 patients with head and neck cancer: 245 received HBO_2 for 30 to 40 treatments; 19.6% recurrence in HBO_2 group vs 28% recurrence in non-HBO_2 group

↑ indicates increased growth; ↓ indicates decreased growth; ↔ indicates no effect on growth. If 2 symbols are given, the effect is mixed.

Tumor angiogenesis has been a hot topic in Oncology over the past decade or so with the delayed popularization of the work of Judah Folkman, M.D. from Harvard. For 30 years, Dr. Folkman[52] has proposed that tumor angiogenesis plays a key role for tumor growth and metastasis and that anti-angiogenic therapies are important and unutilized strategies likely to be effective in tumor control. His work is now widely accepted in principle, and there are currently many different anti-angiogenic factors under study in Phase I, II and III clinical trials.[53] These trials are directed at blocking tumor angiogenesis at one or more points along a complex cascade of events that must come together to allow tumor angiogenesis to progress successfully. Without angiogenesis, tumor growth is restricted to two cubic millimeters or less and metastases will not occur. (Dr.Folkman[54] has estimated that every endothelial cell supports as many as 100 tumor cells).

Steps in the Angiogenesis Process

For tumor angiogenesis to occur a number of coordinated steps must successfully occur.[55]

A. The basement membrane of existing blood vessels must be broken down along with their extracellular matrix. These actions are controlled by a class of enzymes called matrix metalloproteinases (MMP's). The breakdown of the basement membrane allows new branches to form off an existing blood vessel.

B. Once the vascular basement membrane has been breached, endothelial cells must divide to form vascular tubules branching off from the existing blood vessels. Endothelial cell division is regulated by a balance between growth and inhibitory factors. Once endothelial cells have begun to proliferate, they must then join together to form a closed tube.

   i. Over a dozen growth factors have been identified that increase proliferation, survival and motility of endothelial cells. VEGF (Vascular endothelial growth factor) appears to have the most cell specific effect on endothelial cell mitosis. Acidic and basic fibroblast growth factors (aFGF and bFGF), epidermal growth factor (EGF), interleukin-8, and tumor necrosis factor alpha also play a prominent role. Endothelial surface proteins such as $alpha_v$, $beta_3$ integrin and E-selectin increase the motility and survival of endothelial cells.

   ii. Other inhibitory circulating factors regulate endothelial cell mitosis and motility. These include angiostatin, endostatin, interferons alpha and beta, platelet factor 4 (PF4), and thrombospondin-1. Several antagonists of the matrix metalloproteinases have also been identified. These include TIMP-1, TIMP-2 and TIMP-3 (tissue inhibitors of metalloproteinase).

   iii. A final group of factors controls the re-establishment of the integrity of the basement membrane for the newly formed vascular tubules. These are not as well studied but include the angiopoietins (ang-1 and ang-2).

   iv. A group of receptors on the endothelial cells has also been identified with which both the inhibitory and angiogenic factors can interact. These also represent potential targets for disruption of angiogenesis.

Tumor angiogenesis is therefore quite complex involving multiple discrete steps. Each of these may offer a separate potential strategy for disrupting this complex system of tumor vasculature and thus destroying a tumor or at least inhibiting its growth.

Tumor Compartments

Folkman[56] has suggested that in regard to angiogenesis, a tumor can be considered as composed of 2 compartments: 1) The tumor cell compartment and 2) the endothelial cell compartment. Each compartment is highly interdependent and each offers opportunities for therapeutic intervention.

i. The predominant environment of the tumor cell compartment is hypoxic, acidotic and hypoglycemic. Cancer cells divide rapidly and their hypermetabolic activity in poorly vascularized regions leads to anaerobic glycolysis, glucose depletion and lactic acid production. The elaboration and release of mitogenic growth factors including VEGF and bFGF occurs in this compartment. Hypoxia up-regulates VEGF release. These growth factors in turn stimulate rapid proliferation of endothelial cells.

ii. Endothelial cells release growth factors including PDGF, interleukin-6 and IGF-1 (Insulin-like growth factor). These growth factors in turn stimulate proliferation and/or motility of tumor cells.

Angiogenesis in Wound Healing: The Role of Oxygen, a Brief Review

Wound healing like tumor angiogenesis requires complex multi-step interactions between cells, growth factors and extracellular matrix. Angiogenesis is a major component of the wound healing process.[57]

A. The Process of Wound Healing:

Knighton[58] has suggested that the healing wound can also be approached as a 2 Compartment Model:

The wound space is the first compartment and comprises the regulatory compartment. Here, the environment is hypoxic, acidotic, hyperkalemic and hypercarbic. At the edge of the wound near the last perfused capillary, oxygen tensions are in the range of 40mmHg and go to 0 to 15 mmHg at the center of the wound. In this hypoxic environment, from the regulatory compartment a number of growth factors are elaborated that lead to angiogenesis.

i. These growth factors can be grouped into 3 major categories:
   a. Mitogens which signal cells to undergo mitosis.
   b. Chemoattractants that stimulate cells to migrate including macrophages.
   c. Transforming growth factors, which change cellular phenotype.
   Many growth factors are both mitogens and chemoattractants.

ii. Mitogens include platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and angiogenesis factors including acidic and basic ibroblast growth factors (aFGF and bFGF). In the wound space compartment, hypoxia and lactic acid stimulate both growth factor production and macrophage migration. In short order after wounding, macrophages are attracted into the wound space where they perform a dual role: 1) They engulf and destroy bacteria and other cellular debris in the

wound and 2) They release many growth factors including angiogenesis factors. Just as in tumors, these factors must encourage endothelial cell migration, proliferation and basement membrane matrix production after new vascular tubes are formed.

iii. The chemoattractants include complement C5a, which is chemotactic for neutrophils and PDGF, which is chemotactic for fibroblasts.

iv. The final group of growth factors is the transforming growth factors. These growth factors are believed to stimulate production of matrix molecules, i.e. collagen and glycosoaminoglycans. At certain concentrations, they may inhibit fibroblast mitoses.

B. The second compartment is the Responder Compartment, which is composed of vascularized connective tissues and replaces the wound space as the wound heals**.** Here oxygen plays a crucial role in collagen synthesis, hydroxylation and cross-linking. Oxygen is also necessary for epithelization.

Oxygen and Tumor Angiogenesis: What We Know and Surmise

A. Basic Principles: The similarities between tumor angiogenesis and wound healing angiogenesis are striking. Since HBO_2 is promoted in part to stimulate angiogenesis for successful wound healing, should we be concerned that it might also enhance angiogenesis in cancers? Should we not treat a patient with cancer or even a remote history of cancer because we might activate an inactive cancer or its dormant metastases? These are valid questions, and though all of the mechanisms by which HBO_2 might enhance tumor angiogenesis are not known, the information that is available strongly suggests that HBO_2 is not likely to enhance tumor angiogenesis. In fact, we do know tumor cells that grow and survive in hypoxic regions of the tumor are more aggressive, more prone to metastasis and more resistant to treatment. What are the specific considerations?

i. At this point in time, we only partially understand the mechanisms by which angiogenesis is enhanced by oxygen and shut down at the completion of wound healing. HBO_2 does not accelerate healing in well-oxygenated wounds. The growth of malignancies including angiogenesis continues regardless of oxygen status. In other words, tumor angiogenesis is different from angiogenesis in healing wounds in very important ways.

ii. In contrasting and comparing angiogenesis in wounds and malignant tumors Crowther and associates[59] discuss the effects of the microenvironment in both circumstances. They remark on many similarities but also highlight the differences. A major difference they point out is the relative contribution of macrophages in tumor vs. wounds. In wounds, macrophages are the principal source of angiogenic factors; whereas, in tumors macrophages are only one source of these factors, the majority of which come from the tumor cell population and surrounding stroma.

iii. The intermittency of HBO_2 which increases oxygen tensions optimally to the range of 30 to 40 mmHg to stimulate collagen synthesis, hydroxylation and cross-linking appears to be the key in HBO_2 as an adjuvant to healing in chronic hypoxic wounds. No similar mechanisms have been identified in tumor stroma formation.

iv. Angiogenic growth factors elaborated in the wound require hypoxia and lactic acid.[57] Some have suggested that macrophages, a major source of angiogenic factors in wounds, will continue to use anaerobic pathways of glycolysis even in the presence of oxygen at least for some time.[57] It is widely accepted that normal levels of oxygen attained once the wound is healed and vascularized are the signal to discontinue further angiogenesis.[57] It is likely that prolonged exposures to HBO_2 even if tolerable to the patient would have negative effects and ultimately inhibit healing. According to Davis et al[60] in 1988, "Periodic elevation of PO_2 in relatively ischemic wounds has powerful effects on wound dynamics both by enhancing leukocyte bacterial killing and by providing fibroblast-collagen support for capillary angiogenesis factor provided by hypoxic macrophages during the 20-22 hours a day that wound PO_2 drops to hypoxic levels."

Crowther and colleagues[59] reiterate the necessity for hypoxia in promoting angiogenesis in wound healing and state that angiogenesis is markedly decreased when the gradient of oxygen tensions from the center of the wound to the periphery are abolished.

B. Angiogenesis in wounds differ from cancers in several ways:

i. A wound necessarily involves negative space. Even in an approximated surgical wound, the healing process must generate new tissue to occupy this negative space. Tumors generally arise in space already occupied by existing tissues and are characterized by invasiveness. For tumors to grow they must release collagenases to dissolve basement membranes and dissolve normal tissue into which the cancer population of cells can invade and proliferate. Tumors co-opt existing vessels and it is likely that they also co-opt pre-existing stroma.[55] Typically within a tumor mass, the majority of cells composing the stroma and vasculature are themselves not malignant

ii. The substance of the healed wound, i.e. the supporting connective tissues and the overlying epithelium are unlike malignant tumors in that their continued proliferation past healing is regulated by various feedback signals including contact inhibition.[57] In the healed wound unbridled growth is not supported; whereas, it is the nature of malignant cell division that it does not respond to feedback signals from other cells and tissues and that its growth continues unabated regardless of contact by cancer cells to other cells and regardless of oxygen status.[56]

iii. Tumor vasculature is not well organized and does not conform to normal patterns (artery-arteriole-capillary-venule-vein).[56] Tumors often contain giant capillaries and arteriovenous shunts without intervening capillaries. Blood sometimes flows from one vein to another. Leaks in these vessels often contribute to the common phenomenon of peritumoral edema. In other words, tumor angiogenesis does not undergo maturation and integration with pre-existing vasculature in the same fashion as a successfully healed wound.

C. What is known about Tumor Angiogenesis/Growth/Metastasis and Oxygen
   i. Hypoxia has been shown to be an intense stimulus for angiogenesis.[57,58,60]
   ii. VEGF (Vascular Endothelial Growth Factor) has its elaboration and release upregulated by hypoxia not hyperoxia.[59,61-67] Numerous publications have demonstrated the increase of VEGF with hypoxia. In a wide range of tumor types. VEGF is released by the tumor cell itself.[55] A tempting inference (unproven) is that if hypoxia up-regulates VEGF in tumors, hyperoxia should down regulate its release.
   iii. Interleukin-8 release is increased by hypoxia[68] This has been demonstrated in human glioblastoma cells in culture. IL-8 has been shown to have angiogenic properties in this model. The work of Shi and associates[69] confirms an increase in IL-8 by hypoxia and acidosis and suggest this contributes significantly to the aggressive biology of pancreatic cancer.
   iv. PEDF (Pigment Epithelium Derived Factor) an angiogenic inhibitor is down regulated by hypoxia and upregulated by hyperoxia[70]. This effect was demonstrated in human retinoblastoma cells in culture.
   v. Hill and co-workers[71] have reviewed the influence of oxygen status and pH on metastasis. From a murine model with transplanted cells from KHT cells (a fibrosarcoma cell line) and SCC-VII cells (a squamous cell line) when animals were deliberately exposed to cycles of 5% oxygen breathing, they demonstrated a doubling of metastases in the lungs. Niizeki and colleagues[72] report that hypoxia enhances the expression of autocrine motility factor and the motility of pancreatic cancer cells. They suggest that this increase may be the mechanism whereby hypoxia promotes metastases.
   vi. Large scale DNA over-replication and gene (oncogene) amplification occurs in hypoxic regions of tumors.[73] The frequency of mutations in tumor cells in hypoxic conditions was five fold that of cells cultured in normoxic conditions. Teicher[74] has suggested that the genetic instability demonstrated by tumor cells in hypoxic regions is likely to result in the development of drug resistance.
   vii. Hypoxia selects for tumor cells with diminished potential for apoptosis.[75] Apoptosis or programmed cell death is felt to be an important protection against malignancy since malignant cells continue to divide indefinitely. Graeber et al[76] have shown that hypoxia causes defects in apoptosis in oncogenically transformed Rat1 fibroblasts grown in tissue culture.
   viii. Hypoxic tumors are resistant to radiation and some chemotherapy agents.[77,78] Tumors with large populations of hypoxic cells are resistant to killing by ionizing radiation.[77] More recent studies have shown that many chemotherapeutic agents have their efficacy reduced in areas of hypoxia. Teicher et al[78] reported that 3 discrete types of chemotherapies exist in regard to their killing of cells related to the oxygen status of those cells. Type 1 agents demonstrate diminished cell kill in regions of hypoxia; Type 2 agents selectively kill hypoxic cells; Type 3 chemotherapies kill cancer cells equally well in hypoxic and normoxic environments. Type 1

drugs include Bleomycin, Procarbazine, Actinomycin-D and Vincristine. Rice et al[79]have reported that hypoxia leads to resistance to Methotrexate by enhancing the frequency of dihydrofolate reductase gene amplification in Chinese hamster ovary cells.

ix. Hypoxia predicts tumor aggressiveness and metastatic potential. Hoeckel and associates[80] have shown that patients with cervical cancer with significant regions of hypoxia have decreased survival. Gatenby et al[81] reported a higher likelihood of metastases in patients with hypoxic squamous cancers. Brizel and associates[82] reported that patients with larger fractions of hypoxic cells in their soft tissue sarcomas had worse survival and more common metastases than those with higher tumor oxygen levels. For survival the break point was <or> 10 mmHg and for metastases the favorable group had median oxygen values greater than 20 mmHg while the unfavorable group had oxygen levels less than 7.5 mmHg.

x. Vaupel[75] and co-workers reports that hypoxia causes a loss of tumor cell differentiation and apoptosis. They also report that enhanced loco-regional spread, enhanced metastases and resistance to therapy in hypoxic tumors. They show that hypoxia is a powerful independent prognostic factor in cervix cancer, cancers of the head and neck and sarcomas.

Many similarities and important differences exist between tumor and wound angiogenesis. Both require hypoxia for the release of angiogenic growth factors. In wounds, oxygen is needed for its immune effect and to support fibroblast proliferation, collagen release, hydroxylation and cross-linking. Oxygen is also needed for epithelization.[58] Cancers co-opt blood supply initially from surrounding structures and may co-opt stroma as well.[56,57] Certainly, those who have intensely studied tumor angiogenesis have not identified collagen production or release as part of the complex series of events needed to successfully generate tumor angiogenesis. Epithelial coverage is not a major component of cancer growth though it is vital for wound healing. Often cancers become ulcerated and do not have an epithelial cover. The preponderance of tumors show with consistency that hypoxic tumor cells elaborate angiogenesis factors, grow more aggressively, throw off more metastases and are subject to decreased apoptosis and increased genetic instability and therefore increased drug resistance. Hypoxic cells are resistant to irradiation and some chemotherapeutic agents.

CONCLUSIONS

The published evidence suggests that intermittent HBO_2 does not enhance primary or metastatic cancer growth. Likewise, there is no credible evidence that HBO_2 initiates or promotes cancer de novo. Animal studies specifically designed to study the impact of HBO_2 on malignant tumor growth and metastasis conducted from 1966 to 2001 fail in an overwhelming fashion to demonstrate a tumor growth enhancing effect. While 3 clinical publications entailing 72 patients suggest a possible cancer or metastases promoting effect, large numbers of mostly controlled studies including over 3,000 patients enrolled in trials designed to investigate hyperbaric oxygen as a radio-sensitizer demonstrate either a neutral or cancer inhibitory effect. Marx has followed 405 patients treated for delayed radiation injury and observed a decreased incidence of recurrence in those patients treated with HBO_2. The possibility that significant

immune suppression, free radical induced damage or mutations leading to carcinogenesis is likely to enhance malignant growth in hyperbaric patients is not well supported by the reviewed literature. Finally, contentions that tumor angiogenesis is likely to be promoted by HBO_2 in the same fashion that angiogenesis is promoted in non-healing hypoxic wounds fail to recognize the unique nature of those processes in these very different physiologic and pathophysiologic systems. In a wound, macrophages are the principal source of growth factors while in the tumor, the malignant cells themselves elaborate many growth factors. Most recent evidence supports the findings that tumors which thrive in hypoxic environments are more prone to a rapid aggressive course including resistance to treatment, increased incidence of metastases, decreased cell death due to apoptosis and a higher likelihood of tumor lethality. The authors propose that patients for whom HBO_2 treatments are likely to be useful for the treatment of radiation injuries should not have this therapy denied to them because of concerns that HBO_2 might cause a higher likelihood of tumor recurrence or metastases.

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