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Hyperbaric Oxygen Therapy for Malignancy: A Review

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Abstract

One unique feature of tumors is the presence of hypoxic regions, which occur predominantly at the tumor center. Hypoxia has a major impact on various aspects of tumor cell function and proliferation. Hypoxic tumor cells are relatively insensitive to conventional therapy owing to cellular adaptations effected by the hypoxic microenvironment. Recent efforts have aimed to alter the hypoxic state and to reverse these adaptations to improve treatment outcome. One way to increase tumor oxygen tensions is by hyperbaric oxygen (HBO) therapy. HBO therapy can influence the tumor microenvironment at several levels. It can alter tumor hypoxia, a potent stimulus that drives angiogenesis. Hyperoxia as a result of HBO also produces reactive oxygen species, which can damage tumors by inducing excessive oxidative stress. This review outlines the importance of oxygen to tumors and the mechanisms by which tumors survive under hypoxic conditions. It also presents data from both experimental and clinical studies for the effect of HBO on malignancy.

Hyperbaric oxygen (HBO) therapy involves the intermittent administration of 100% oxygen at high pressure. HBO increases oxygen tensions and oxygen delivery to tissues independent of hemoglobin. HBO promotes new vessel growth in poorly perfused areas and has been used to treat a variety of conditions including wounds,[2–4] carbon monoxide poisoning,[5–9] and necrotizing soft tissue infections.[10] HBO has also been used in combination with drugs for the treatment of malignancy.[11–16] This is based on the rationale that tumors may become sensitized to irradiation and other forms of therapy by increasing the intratumoral oxygen tension.

Although most of the experimental and clinical studies suggest that HBO has no direct effect on tumors, there is a considerable amount of conflicting evidence to support the idea that HBO has an effect. The most convincing effects of HBO are observed when it has been used in an adjuvant setting with certain types of malignancy. HBO therefore remains ineffective as a stand-alone therapy or even as a reliable adjuvant. However, HBO may enhance the efficacy of certain therapies that are limited because of the hypoxic tumor microenvironment. Further research should also consider treatment feasibility and economic expenditure. These factors should be weighed against potential therapeutic benefit before HBO can be given credit in the treatment of malignancy.

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TUMOR BIOLOGY

Tumor Hypoxia

Growth of tumors is limited by the delivery of oxygen and nutrients and the removal of waste products. Oxygen and nutrients are initially delivered to tumor cells by diffusion from the surrounding microenvironment. As a tumor grows, cells undergo nutrient deprivation and acidosis, and they become hypoxic. Moderately sized

experimental tumors, 4 to 10 mm in diameter, exhibit large regions of hypoxia.[17,18] This results in decreased oxygen tensions (< 20 mmHg) that approach 0 mmHg at the tumor center, leading to central necrosis.[19] Oxygen tension in tumors ranges from 2.5 to 30.0 mmHg. This is in contrast to normal tissue and the tumor periphery where oxygen tensions are between 30 and 60 mmHg.[19,20] Three levels of oxygenation coexist in tumors: normoxic (tumor periphery and groups of cells in a tumor mass), hypoxic (adjacent to regions of necrosis distant from blood vessels), and anoxic (tumor center). This makes the tumor microenvironment toxic.

Hypoxic tumor cells survive by adapting to the adverse conditions, and are a potential source of tumor recurrence and treatment failure in several forms of malignancy.[21–25] Tumor hypoxia can limit the efficacy of therapy in several ways. Malignant cells in hypoxic regions are exposed to lower drug concentrations owing to the limited access of intravenously administered drugs to avascular regions. Hypoxic cells undergo arrest and enter a nonproliferating state. A study conducted by Cuisnier *et al.* on squamous cell carcinoma cells in culture showed a 20% increase in the number of cells in G0/G1 arrest when exposed to chronic hypoxia compared to normoxic cells.[26] These cells are less susceptible to chemotherapy and radiotherapy, which target rapidly proliferating cells. Furthermore, hypoxia directly affects the expression of many gene products that are involved in angiogenesis, apoptosis, and glycolysis. Surviving malignant cells are preferentially selected and undergo clonal expansion, giving rise to a highly malignant cell line. This culminates in a more aggressive phenotype associated with poor patient survival as seen in patients with cancer of the uterine cervix,[23,27–29] squamous cell carcinoma of the head and neck[21] and renal and bladder cancer.[30]

Tumor cells adapt to the ischemic and low nutrient microenvironment by three main adaptations. First is the *angiogenic switch*, which results in a shift in balance of proangiogenic versus antiangiogenic factors leading to the formation of an aberrant vascular network. Second is *deregulation of apoptosis*, where critical components of the apoptotic cascade are altered allowing tumor cells to evade apoptotic destruction. Third is the *glycolytic shift*, where tumor cells preferentially switch to anaerobic glycolysis. All three mechanisms are driven by the hypoxic tumor microenvironment.

Angiogenic Switch

Successful growth and metastases of tumors requires the establishment of an efficient blood supply. Tumor cells produce proangiogenic growth factors to initiate the formation of new blood vessels. This is known as the *angiogenic switch* involving up-regulation of proangiogenic factors and down-regulation of angiogenic inhibitors. The resultant tumor microvasculature is highly disorganized and contains many tortuous vessels that are irregular in diameter. These vessels have heterogeneous permeability and are functionally abnormal.[31,32] Large pools of coalesced vessels interspersed with avascular areas lead to regions of stagnant or intermittent blood flow.[33] This results in a highly inefficient, variable, and greatly reduced blood supply compared to normal vasculature, which leads to further hypoxia.

The most potent stimulus for angiogenesis is metabolic stress induced during hypoxia. Proangiogenic factors, which are secreted by tumor cells, surrounding endothelial cells, or infiltrating inflammatory cells, are involved in endothelial cell invasion, migration, and survival. Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), and the angiopoietin families are involved in the development and differentiation of the vascular system.[34] High levels of circulating plasma VEGF have been correlated with a poor prognosis in several cancers including breast,[35] prostate,[24,36] pancreatic,[37] renal,[38] head and neck,[22] and colorectal cancer.[39]

The most commonly expressed cytokine, VEGF is induced by hypoxia.[40] It is a multifunctional cytokine that increases microvascular permeability and induces endothelial cell migration by promoting the invasion of collagen by vascular endothelial cells, resulting in the formation of tube-like structures.[41,42] The resultant vessels are abnormal with chaotic blood flow, resulting in hypoxia, which in turn drives further angiogenesis. This acts as a positive feedback mechanism (Fig.1, A). Hypoxia is thus both a cause and a consequence of angiogenesis.

In vitro[43,44] and in vivo[24,45] evidence supports hypoxia as a potent stimulator of VEGF expression.[46] VEGF mRNA has been shown to either co-localize with hypoxic cells or localize adjacent to hypoxic cells.[47] The VEGF gene has been shown to be particularly active under hypoxic conditions at the level of transcription, increased stability of VEGF mRNA, and preferential translation.[40,48–50] A study conducted in human tumors found that in all tumors highly expressing VEGF the mRNA signal pattern is highly correlated with hypoxia as determined by binding of the hypoxia marker EF5.[45] Increased VEGF mRNA and protein levels were also found in hypoxic brain tissue compared with normoxic tissue.[51] A study conducted on ovarian cancer cells showed

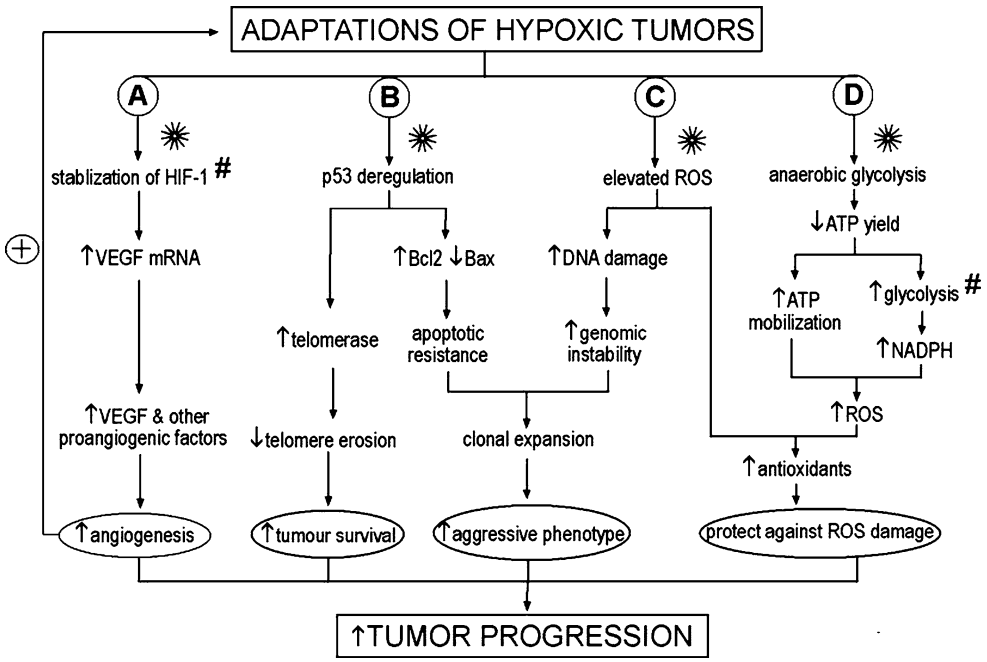

Figure 1. Tumors survive under hypoxic conditions via adaptations that are regulated by the hypoxic microenvironment. These adaptations facilitate tumor progression by re-regulating molecular mechanisms involved in angiogenesis and survival. HBO may interfere with each adaptation (*) by altering the hypoxic state of tumors. #Increased glycolysis promotes expression of hypoxia inducible factor-1 via stabilization of hypoxia inducible factor-1α (HIF-1). VEGF: vascular endothelial growth factor; ROS: reactive oxygen species; ATP: adenosine triphosphate; NADPH: reduced nicotinamide adenine dinucleotide phosphate.

increased VEGF expression as a consequence of hypoxia via up-regulation of hypoxia inducible factor (HIF)-1α.[43,45]

Transcriptional activation of VEGF is achieved by the transcription factors HIF-1 and HIF-2. HIF-1 is a heterodimeric transcription factor that regulates oxygen homeostasis and physiological responses to oxygen deprivation. HIF-1 consists of two subunits (HIF-1α and HIF-1β) and is up-regulated as a result of decreased cellular oxygen tensions via stabilization of the HIF-1α protein.[43,52] Under normoxia HIF-1α is degraded by hydroxylases, but under hypoxia HIF-1α evades degradation and dimerizes with HIF-1β. Reexposure to a normoxic environment has been shown to result in rapid decay of HIF-1 activity.[53] Similarly, loss of HIF-1α in endothelial cells results in profound inhibition of blood vessel growth in solid tumors[54] and causes vascular regression in HIF-1α-deficient mouse embryos.[52] This shows that HIF-1 is a key transcriptional mediator of VEGF-induced angiogenesis in response to hypoxia[55,56] (Fig. 1, A). HIF-2 is responsible for vascular remodeling following angiogenesis and also regulates the expression of VEGFR-2 and the VEGF receptor Flk-1.

There are a number of other proangiogenic factors that are up-regulated during the angiogenic switch including platelet-derived growth factor (PDGF), fibroblast growth factor (FGF),[57] angiogenin,[58] epidermal growth factor (EGF), nitric oxide synthase, transforming growth factor-β (TGF-β). The proangiogenic cytokines also play a role including granulocyte/macrophage colony-stimulating factor (GM-CSF) and interleukin-8 (IL-8). All of these factors are elevated in the presence of hypoxia.

Aberrant IL-8 expression has been reported in several solid malignancies including breast,[59,60] colorectal, and pancreatic cancers.[25] Oxidative stress induced by the oxygen radical generating sugar thymidine phosphorylase resulted in induction of IL-8 along with increased VEGF and matrix metalloproteinase-1 (MMP-1).[61] Hypoxia also induced IL-8 mRNA and protein expression in the most aggressive human melanoma cells in vitro.[62] The presence of IL-8 has also been implicated in the production of FGF-2.

FGF-2 is a potent growth factor in prostatic epithelial and stromal tissue as has been shown in prostatic cells.[63] Kuwabara and colleagues showed that macrophages exposed to low oxygen tensions secreted PDGF and FGF, which further stimulated proliferation of hypoxic endothelial cells.[57] Angiogenin, when bound to its receptor, facilitates endothelial cell digestion of the extracellular matrix and degradation of the basement membrane, promoting endothelial cell migration and angiogenesis. High levels of the angiogenin protein and mRNA have been found in

highly aggressive human melanoma cells in vitro and in vivo under hypoxic conditions.[58] The same group showed that only angiogenin and VEGF were up-regulated, and other growth factors tested (e.g., bFGF, PDGF, TGFβ) in parallel showed minimal elevation. Epidermal growth factor (EGF), when bound to its receptor EFGR, results in increased cell proliferation. Increased expression of EFGR has been associated with a worse prognosis and reduced response to chemotherapy in gastric,[64] colorectal,[65] cervical[66] cancer and has been used as a prognostic marker for patients with bladder cancer.[67] In addition to angiogenesis promoting tumor growth and progression, malignant cells also acquire the potential to evade apoptotic destruction.

Deregulation of Apoptosis

To cope with the hypoxic microenvironment, tumor cells increase their metabolic rate, which often leads to DNA damage.[68] Under physiological conditions, when cellular repair enzymes cannot correct the DNA damage, the apoptotic cascade is activated, resulting in cell death. Tumors, on the other hand, possess cellular mechanisms (particularly active under hypoxic conditions) that allow them to evade apoptosis despite the extent of DNA damage. Spontaneously regressing tumors[69] and tumors responding to cytotoxic therapy exhibit a high degree of apoptosis. These mechanisms involve deregulating cellular components and genes critical to cell replication and apoptosis, such as the *p53* tumor suppressor gene. The p53 protein plays a pivotal role in cell cycle regulation and is a promoter of apoptosis.[68,69] It is the most commonly mutated gene in human cancer and correlates with advanced tumor stage and indicates a poor patient prognosis.[70–73] Deregulation of *p53* in cancer occurs through both inactivation of wild-type *p53* and accumulation of mutated *p53*. Loss of *p53* function increases tumor cell viability, chromosomal instability, and cellular life-span.

Telomere length limits the replicating ability of cells, resulting in cell senescence. Developing cells undergo telomere erosion as a result of rapid division. Once the telomeres have eroded, the cell becomes senescent or undergoes apoptosis via activation of *p53*. Replicative senescence or irreversible cell cycle arrest limits the proliferation of damaged cells and is an important tumor suppression mechanism. When DNA is damaged, wild-type *p53* has the ability to induce cell cycle arrest and, if irreversible damage has occurred, induce apoptosis. Mutated *p53* results in reduced telomere erosion via activation of the enzyme telomerase (Fig. 1, B). Telomerase enhances the proliferative capacity of cells by using its own RNA as a template to add telomeric repeats onto the ends of chromosomes. Telomerase is expressed in tumor cells of patients with colorectal,[74] ovarian,[75] gastric,[76] and lung[77,78] cancer. This results in the uncontrolled replication of malignant cells with acquired genomic instability.[68,71–73] Minamino et al. showed that telomerase is particularly up-regulated under chronic hypoxia.[79] Hypoxia also disrupts the regulation of other genes associated with apoptosis.[68,80]

Members of the *Bcl-2* family act as inhibitors (Bcl-2, Bcl-XI, Bcl-W) and promoters (Bax, Bad, Bak, Bcl-Xs) of apoptosis. Alterations in the ratio of these protein expressions may attenuate an antiapoptotic effect. Bcl-2 is a potent inhibitor of cell death that is particularly up-regulated in some tumors, especially in the presence of hypoxia.[81] This allows Bcl-2 to promote tumor cell survival by blocking programmed cell death.[82] Conversely, Bax, a death promoter, is inactivated in certain types of colon cancer.[83] The *Bax* gene, known to promote apoptosis is mutated in several forms of cancer. Overexpression of Bcl-2 combined with loss or mutation of *Bax* and *p53* causes a significant reduction in the apoptotic capacity of cells (Fig. 1, B).

These adaptations are integrated to some extent. Deregulation of apoptosis can influence angiogenesis. A study conducted on colorectal tumor xenografts in nude mice found that deletion of *p53* promoted neovascularization of tumors through enhanced HIF-1 levels, which augmented the expression of VEGF.[80]

Chronic hypoxia induced neither apoptosis nor necrosis of the KB-3-1 head and neck squamous cell carcinoma cell line due to an imbalance in the ratio of Bcl-2/Bax.[26] Hypoxic regions have also been correlated with reduced apoptotic potential of tumors with a highly malignant phenotype.[23,68] The cellular responses described above are adaptations tumor cells make as a result of oxygen deficiency. Hypoxia hereby acts as a physiological selective agent promoting the clonal expansion of a highly aggressive lineage of cells with a range of cytogenetic abnormalities that are resistant to apoptosis.

Cells in a hypoxic microenvironment are deprived not only of oxygen but also of nutrients. The third adaptation tumor cells make in response to hypoxia and nutrient deprivation is to attain energy from an alternate pathway, known as the *glycolytic shift*.

Glycolytic Shift

As a result of increased energy demands amidst a diminished oxygen supply, tumors depend on anaerobic

glycolysis. The switch to anaerobic glycolysis is an important adaptation facilitating rapid tumor progression and is known as the *glycolytic shift*. This was first proposed by Otto Warburg and is termed the ''Warburg effect''; it results in a shift in energy production from oxidative phosphorylation to anaerobic glycolysis.[84]

Glycolysis is a universal metabolic pathway for the catabolism of pyruvate accompanied by the formation of adenosine triphosphate (ATP), which is the main source of energy for cells. The glycolytic pathway is regulated by key enzymes beginning with glucose entering the cell bound to a glucose transporter, either GLUT-1 or GLUT-3. The first phosphorylation is catalyzed by the enzyme hexokinase. Under aerobic conditions pyruvate is metabolized to form carbon dioxide and water, resulting in a high ATP yield. Under hypoxia, insufficient oxygen is available to support the aerobic oxidation of pyruvate. Instead, anaerobic glycolysis occurs where pyruvate is reduced to lactate resulting in a low ATP yield. To compensate for the low ATP yield, tumors increase their glycolytic rate.[1] Increased glucose metabolism produces nicotinamide adenine dinucleotide (NADH), which is constantly reoxidized to sustain continual anaerobic glycolysis.

The glycolytic shift is beneficial for tumors at several levels and primarily occurs under the influence of the transcriptional factor HIF-1 and other cell signaling mechanisms driven by hypoxia. As discussed earlier, HIF-1 regulates numerous genes involved in angiogenesis,[55] cell cycle control,[43] and glycolysis,[52] including GLUT-1 and hexokinase.[85,86]

Hypoxia elevates the expression of many key glycolytic enzymes, as shown by Webster et al. in partially differentiated mammalian myotubes in vitro under normoxic and hypoxic conditions. Under hypoxic conditions, the glycolytic enzyme mRNAs increase and the respiratory mRNAs (involved in oxidative phosphorylation) decrease. The inverse occurred under normoxic conditions.[87] Overexpression of the glucose transporter genes GLUT-1 and GLUT-3 has been observed in human tumors.[85,88] HIF-1 also up-regulates mitochondria-bound hexokinase.[89,90] This enzyme is involved in the first phosphorylation of glucose during glycolysis and is unresponsive to feedback inhibition. This commits the tumor cell to continued glycolysis (Fig. 1, D). There is also evidence of the end-products of glycolysis, such as pyruvate promote stabilization of the HIF-1α protein and activated HIF-1 gene expression,[91] thereby facilitating further glycolysis and tumor progression (Fig. 1, #).

In addition to HIF-1, the mutated oncogenes *p53* and *myc*[92] and defects in cell signaling such as the Akt kinase pathway[93] have also been shown to increase the glycolytic capacity of tumor cells. Increased glycolysis places tumors under constant oxidative stress for several reasons.

Chronic exposure of cells to high glucose levels has been shown to increase intracellular reactive oxygen species (ROS) production.[94] Due to the low ATP yield from glycolysis, ATP is generated from alternate sources such as the degradation of cellular proteins and amino acids. This degradation results in production of ROS, which induces further oxidative stress (Fig. 1, D). Increased glycolysis results in the production of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a primary inducer of the superoxide and catalase radicals.[95] To ensure that the ROS content does not reach toxic levels, antioxidant defenses are switched on. The antioxidant glutathione peroxidase is produced. It has been shown that production of this antioxidant is sustained by NADPH produced as a result of increased glycolysis.[96,97]

The glycolytic shift provides a pathway for tumors to sustain an increased metabolic rate under hypoxia. More importantly, this adaptation induces stabilization of HIF-1, which regulates processes that not only maintain glycolysis but facilitate tumor progression. Transcription of VEGF and IL-8 are up-regulated in tumor cells in response to glucose deprivation via HIF-1-dependent mechanisms. This is an example of how one adaptive mechanism (glycolytic shift) sustains another mechanism of adaptation (angiogenesis) to promote tumor growth.

ROS Production

Reactive oxygen species, or free radicals, are a by-product of aerobic respiration and cellular metabolism and are produced by all eukaryotic cells. Low levels of ROS are generated in the mitochondria and are important for regulating signal transduction and normal cell proliferation and function.[98,99] ROS include the superoxide anion ($^{\cdot}O_2^-$), hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). The main cellular components susceptible to damage by ROS are lipids, proteins, carbohydrates, and nucleic acids.[100] In excess, ROS cause lipid peroxidation, compromise cell membrane integrity, and lead to cell death. Excess ROS also cause DNA strand breaks, resulting in mutations or deletions of various genes.[101,102]

There is substantial evidence for the involvement of ROS in carcinogenesis.[99,102,103] ROS accumulation has been implicated in the initiation and progression of tumors.[101,104] ROS are induced by oxidative stress during oxygen deficiency, reoxygenation (reperfusion), or

excess oxygen (hyperoxia). Levels of ROS are tightly regulated by antioxidant defenses, which prevent oxidative damage. During oxidative stress, the antioxidants superoxide dismutase (SOD), catalase, glutathione peroxidase, and bilirubin are up-regulated in tumors[103] compared to normal tissue.[105]

The production of ROS in normal and tumor tissue is fundamentally different. In nonmalignant cells, ROS levels are relatively low and tightly regulated by antioxidants.[99,106] In contrast, tumors are under constant oxidative stress due to increased glycolysis, transcription factor activation, and vascular architecture. The chaotic and erratic blood flow of tumors results in intermittent periods of hypoxia followed by reperfusion. Reperfusion following myocardial infarction or cerebral ischemia is known to cause generation of ROS. Based on the same concept, oxidative stress induced by reperfusion is a major source of ROS production in tumors.[70] ROS production also mitigates cell signaling, which has been shown to promote oncogenic transformation and uncontrolled proliferation.[107] Markers of oxidative stress have been detected in samples from in vivo breast carcinomas, and human tumor cell lines in vitro have been shown to produce more ROS than nonmalignant cell lines.[70]

Elevated ROS offer a selective growth advantage to tumor cells in several ways. ROS are responsible for DNA strand breaks, leading to mutations in tumor cells. These mutations may affect the genes responsible for apoptosis or induce oncogenic transformation of cells.[108,109] ROS promote the constant activation of transcription factors, leading to increased proliferation of cells with acquired DNA damage. These cells are genomically unstable owing to intrinsic mutations and contribute to a more aggressive phenotype (Fig.1, C).[102]

Upon exposure to some anticancer agents, intratumoral ROS become greatly elevated. Initially this oxidative stress triggers apoptosis. In advanced cancer however, adaptive mechanisms such as evasion of apoptosis or up-regulation of antioxidants prevent destruction of these cells which then undergo clonal expansion[99] (Fig. 1, C). Antioxidants such as SOD and glutathione peroxidase are markedly up-regulated in several forms of cancer.[110]

Prolonged exposure to anticancer agents leads to apoptosis via excessive ROS generation, which occurs when the antioxidant system is overwhelmed. This can be explained by the ''threshold effect'' whereby ROS reach a level beyond which the antioxidant capacity is inundated, resulting in irreversible damage and apoptosis.[106,111–113]

To ensure ROS do not exceed the threshold level, tumors induce rapid up-regulation of antioxidants such as SOD,[114] glutathione peroxidase, catalase,[115] and bilirubin,[116] which have all shown increased expression and activity in tumors compared to normal tissue. Evidence supporting the threshold effect is provided by clinical studies where increased H_2O_2 levels result in proliferation of normal cells and destruction of tumor cells, whereas when H_2O_2 is decreased, the reverse occurs. This supports the idea that ROS levels in tumors are normally at sublethal doses, and any increase would induce cytotoxicity.[106,111–113]

The concept of the threshold effect may be an attractive therapeutic approach to destroying tumors with persistent ROS production.[103,117–119] The nature of ROS in tumors is therefore paradoxical. Although their accumulation leads to cancer initiation and sustained progression, they may also serve as a target for therapy. Therapies that induce ROS production include the chemotherapeutic agent doxorubicin, an O_2^--generating agent, and bleomycin. Radiotherapy and photodynamic therapy also induce ROS generation in tumors.

Oxygen deficiency limits treatment efficacy on several fronts. First, chemotherapeutic drugs are unable to reach all tumor cells in a poorly perfused microenvironment. Administration of a higher dose is not an option because of the severe dose-limiting side effects. Radiotherapy destroys tumor cells only in well oxygenated regions. Second, hypoxia induces cell cycle arrest so some tumor cells become trapped in the G0/G1 phase and remain noncycling.[120] In addition to this, hypoxia drives angiogenesis-promoting tumor growth and metastases under oxidative stress. Tumor cells under oxidative stress produce ROS, which result in mutations. Deregulation of the apoptotic cascade in the presence of hypoxia prevents malignant cell destruction. The selective replication of these defective cells leads to genomic instability. All of these events culminate in a highly aggressive tumor in which regions of cells are resistant to destruction and can cause tumor recurrence. Novel strategies target tumors by altering the hypoxic state through improved oxygenation to possibly reverse or remove the adaptive defenses of tumors or induce oxidative stress to promote tumor destruction. The latter strategy has been investigated on human colon and liver carcinoma cell lines in vitro.[106] Improving tumor oxygenation and vascularization may increase drug delivery. This has been shown experimentally[121] and in nude mice with human epithelial ovarian cancer treated with cisplatin.[122] It may also reduce or remove the hypoxic stimulus that triggers the adaptive mechanisms of tumors. One way to improve tumor oxygenation is to administer hyperbaric oxygen.

Table 1.
Animal studies for the effect of HBO on malignancy

Study	Year	Animal model	Animal tumor	HBO regimen
De Cosse[14]	1966	Syrian hamsters (n = 160)	Melanoma	2.0 atm 7–12 exposures, 6 days
McCredie[143]	1966	C3H mice (n = 282)	C3HBA murine tumor	3.0 atm 12 exposures 30 minutes
Suit[144]	1966	BDF mice	Mammary tumor	3.0 atm 30 exposures 60 minutes
Johnson[145]	1967	CDBA (F_1) mice (n = 350)	Melanoma and leukemia	3.0 atm 20 exposures 30 minutes
Dettmer[149]	1968	CFN albino rats (n = 60)	Walker carcinosarcoma	1.0 and 3.0 atm8–15 exposures
Feder[146]	1968	C3H mice (n = 418)	C3H rhabdomyo-sarcoma	3.0 atm 20 exposures 20 minutes
Valaitis[139]	1968	Swiss mice (n = 600)	Ehrlich ascites tumor	2.0 atm 7 exposures 120 minutes
Evans[161]	1969	CBA mice (n = 245)	Squamous skin carcinoma	2.0 atm 1 exposure
Johnson[147]	1971	DBA/2 mice (n = 92)	Lymphoblastic leukemia	3.0 atm 11 exposures 90 minutes
Shewell[137]	1980	C3H/Bts mice (n = 44)	Transplanted and spontaneous mammary tumors	3.0 atm 6 exposures 20 minutes
Martin[162]	1987	WAG/rij-Y rats	BA1112 rhabdomyosarcoma	3.0 atm 30 minutes
Marx[131]	1987	Hamster	DMBA-induced SCC	2.4 atm 20 exposures
Frid[163]	1989	SHR miceC57/B1 mice	Transplanted sarcoma 37 Melanoma B16	Not reported
McMillan[138]	1989	Syrian hamsters (n = 30)	DMBA-induced oral mucosal SCC	2.5 atm 85 exposures 99 minutes
Granstrom[164]	1990	C-57 mice	Sarcoma	2.8 atm 9 exposures 120 minutes
Mestrovic[148]	1990	Y59 rats (n = 38) (n = 16)	Anaplastic CA-induced lung metastases Anaplastic CA in hind foot	1.0 or 3.0 atm 16 exposures 90 minutes
Headley[165]	1991	Nude mice	Human SCC xenografts	2.4 atm 15 exposures
Sklizovic[166]	1993	Nude mice (n = 40)	Human head and neck SCC	2.0 atm 21–28 exposures
Lian[135]	1995	ICR mice (n = 120)	S-180 murine sarcoma	2.5 atm 18 exposures 90 minutes
McDonald[167]		Syrian hamsters (n = 40)	DMBA-induced tumors	2.8 atm 30 exposures 60 minutes
Takiguchi[15]	2001	DDY mice (n = 41)	Sarcoma 180	2.0 atm 17 exposures 90 minutes
Huang[6]	2003	C3H mice	C3H tumors	3.0 atm 15 minutes
Petre[12]	2003	Sprague-Dawley rats (n = 24)	MCA2 sarcoma	2.0 atm 7 exposures 30 minutes
Shi[168]	2005	Ncr-nu/nu mice	Head and neck SCCA	2.4 atm 13-28 exposures 90 minutes

HBO: hyperbaric oxygen; atm: atmospheres; PDT: photodynamic therapy; SCC: squamous cell carcinoma; CA: cancer; DMBA: dimethylbenzanthracene; N/A: survival not assessed.

+: HBO had a tumor stimulatory/adverse effect; –: HBO had a tumor inhibitory effect; 0: HBO had no effect on tumors. If two symbols are given, the effect was mixed.

Additional therapy	Outcome	Comment	Survival
Mechlorethamine, cyclophosphamide + amethopterin	–	HBO alone decreased pulmonary metastases HBO had no synergistic effect on primary tumor growth	Increased survival ($P < 0.001$)
None	0	No effect on primary tumor No effect on lung metastases 50 days after excising primary tumor	N/A
None	0	No effect on primary or metastases	N/A
None	0	No effect on primary tumor size or number of metastases	Leukemia No effect on survival
None	–	HBO significantly reduced distribution and number of distant metastases	N/A
None	0	No effect on metastases	N/A
Nitrogen mustard (HN_2)	+	Increased tumor growth and metastases HBO appeared to act synergistically with HN_2	HBO alone decreased survival
Radiotherapy	0	No effect on lung metastases with radiotherapy and HBO ($P > 0.1$)	N/A
None	0	No effect on tumor weight, growth rate, or metastases ($P = 0.5$).	No effect on survival ($P = 0.05$)
None	0	No effect on transplanted primary tumors	N/A
	+	Higher incidence of lung metastases in spontaneous tumors in HBO group (88.8%) vs. control (66%)	N/A
Fluosol-DA and radiotherapy	–	Pretreatment with HBO and Fluosol significantly reduced tumor cell survival	N/A
None	–	HBO delayed tumor growth	N/A
None	0	No effect on growth or metastases of transplanted tumors	N/A
None			
None	–	HBO reduced number of tumors ($P < 0.01$). HBO increase tumor size ($P < 0.02$)	N/A
None	0	No effect on tumor growth	No effect on survival
None	–	1 atm HBO had no effect on tumor growth3 atm HBO strongly suppressed lung metastases ($P < 0.001$) No effect on tumor growth.	Increased survival ($P < 0.01$)
None	0	No effect on tumor growth	N/A
None	0	No effect on tumor growth, volume ,or histology	N/A
None	–	Reduced tumor volume in HBO group compared to control ($P < 0.01$) Increased necrosis in HBO group (33%) compared to control group (17%)	HBO benefited survival 26.7%, vs. control 6.7%.
None	–	HBO significantly reduced tumor size ($P < 0.05$)	N/A
5-Fluorouracil (5-FU)	–	Combined HBO + 5-FU resulted in greatest tumor reduction ($P < 0.01$).	N/A
PDT	–	HBO significantly improved tumor oxygenation and tumor cell kill of PDT	N/A
Doxorubicin	–	Combined therapy significantly reduced lung metastases ($P < 0.01$) and overall lung weight ($P < 0.01$) compared to doxorubicin alone	N/A
None	0	HBO had no effect on tumors	N/A

Table 2.
Clinical trials for the effect of HBO on malignancy

Study	Year	Patients (no.)	Trial type	Tumor	HBO regimen
Johnson[136]	1966	25	Uncontrolled	Advanced cervical CA	3.0 atm 30 exposures
Cade[140]	1967	49 40	Randomized control trial	Bronchogenic CA Bladder CA	3.0 atm 40 exposures, < 40 minutes
Van den Brenk[151] Radiotherapy	1967	85 51	Controlled trial	Advanced head and neck Misc. CA (breast, bladder bowel, uterus)	3.0 atm 2–6 exposures
Johnson[169]	1974	64	Controlled trial	Cervical CA	3.0 atm 25–30 exposures
Bennett[170]	1977	213	Controlled trial	Cervical SCC	3.0 atm 10 exposures
Henk[11]	1977	276	First controlled trial	Head and neck CA	3.0 atm 10 exposures
Henk[171]	1977	104	Second controlled trial	Head and neck	3.0 atm 10 exposures
Dische[152]	1978	1500	Controlled trial	Head and neck, bladder, bronchus, or cervical CA	3.0 atm 6–12 exposures
Perrins[78]	1978	236	Controlled	Bladder CA	3.0 atm 6–40 exposures
Watson[172]	1978	320	Controlled trial	Cervical CA	3.0 atm6–27 exposures
Brady[173]	1981	65	Controlled trial	Cervical SCC	3.0 atm 10–12 exposures
Henk[154] et al. [154].	1986	104	Prospective controlled trial	Head and neck SCC	4.0 atm 10 exposures
Sealy[155]	1986	130	Prospective randomized trial	Head and neck SCC	3.0 atm 6 exposures
Eltorai[141]	1987	3	Anecdotal report	2 Bladder and 1 urothelial CA	2.0 atm 10–20 exposures
Bradfield[150]	1996	4	Anecdotal report	Head and neck SCC	Pressure not reported 8–14 exposures
Granstrom[174]	1996	123	Prospective trial	Head and neck	2.5 atm 30–90 exposures
Dische[175]	1999	335	Randomized controlled trial	Advanced cervical SCC	3.0 atm 10 exposures
Haffty[176]	1999	48	Randomized trial	Head and neck SCC	4 atm 2 exposures
Haffty[177]	1999	45	Retrospective trial	Laryngeal CA	4 atm 2 exposures
Kohshi[178]	1999	29	Nonrandomized trial	Glioblastoma	2.5 atm 20–30 exposures
Maier[157]	2000	75	Prospective nonrandomized trial	Advanced esophageal CA	2.0 atm 1–3 exposures

MCA: methylcholanthrene; Gy: Gray (1 gy = 100 rad); fx: fractions; N/A – survival not assessed
+: HBO had a tumor stimulatory/adverse effect. –: HBO had a tumor inhibitory effect; 0: HBO had no effect on tumors. If two symbols are given, the effect was mixed.

Additional therapy	Outcome	Comments	Survival
Radiotherapy	+	HBO group had early appearance and unusual frequency and pattern of metastases	N/A
Radiotherapy	0	No effect on primary tumor growth or metastases	No effect on survival
	+	Enhanced tumor development and doubled metastases	HBO decreased survival
Radiotherapy			
Radiotherapy	–	Fewer metastases with HBO (41%) vs. control (68%) ($P < 0.05$). Significantly decreased metastases ($P < 0.014$)	No effect on survival
Radiotherapy			
None	0	No effect on metastases	HBO improved 5-year survival (44% vs. control 16%)
Radiotherapy	0	Combined treatment with HBO increased local clearance rate but had no effect on metastases	No effect on survival
Radiotherapy 35 Gy/10 fx	0	Improved local tumor control in combined group Reduced need for salvage surgery ($P < 0.01$)	No effect on survival
Radiotherapy	–	Local recurrence free rate better in HBO group	Statistically improved disease-free survival in HBO group
Radiotherapy	0	HBO reduced recurrence ($P < 0.001$) but had no effect on metastases ($P = 0.97$)	Improved 5-year survival ($P < 0.001$)
Radiotherapy	0	HBO had no effect on hypoxic tumor cells or metastases	No effect on survival at 4 years ($P = 0.68$)
Radiotherapy	0	No effect on metastases Increased recurrence-free rate with combined therapy only in patients < 55 years ($P < 0.001$)	No effect on survival
Radiotherapy	0	Distant failure higher in control group 34% vs. HBO group (16%)	No effect on survival
Radiotherapy 35 Gy/10 fx	–	Improved local control of tumors and less advanced tumors	5-Year survival 60% for combined therapy vs. 30% for control
Radiotherapy 36 Gy/6 fx + misonidazole 63 Gy/30 fx in air	–	Combined therapy improved local tumor control by 15%	N/A
–	+	Aggressive tumor growth after HBO therapy	N/A
Radiotherapy	+	Rapid progression of tumors and increased tumor recurrence after HBO therapy	HBO did not improve survival
Radiotherapy	–	Recurrence rate 16% lower in combined therapy group	N/A
Radiotherapy	0	Combined therapy did not improve local tumor control. Some late morbidity with HBO observed	No effect on survival
Radiotherapy 23 Gy/2 fx with HBO 25 Gy/ 2 fx in air	–	Significantly improved 5-year local tumor control at both radiotherapy doses	No effect on survival
Radiotherapy 22 Gy/2 fx with or without HBO	–	Complete response in 87% of casesImproved local 10-year control in most responders	N/A
Radiotherapy 57.8 Gy with or without HBO	–	73% Tumor regression in half of responders	Median survival 24 months in combined group vs. 12 months ($P < 0.05$)
PDT	–	Combined therapy reduced tumor length ($P = 0.0002$)	HBO improved survival ($P = 0.0098$)

MODIFICATION OF TUMOR HYPOXIA WITH HYPERBARIC OXYGEN THERAPY

Hyperbaric oxygen (HBO) therapy involves the administration of pure oxygen at a pressure greater than 1 atmosphere (atm).[13,123] At normal atmospheric pressure (1 atm), hemoglobin is approximately 97% saturated with oxygen. This is equivalent to 19.5 volume percent (vol%) oxygen. Approximately 0.32 vol% of oxygen is dissolved in plasma. Any further increase in oxygen pressure or concentration has minimal impact on total hemoglobin oxygen saturation. Most HBO treatments are performed at a pressure of 2 to 3 atm. The additional pressure when coupled with inspiration of 100% oxygen substantially increases the amount of oxygen dissolved in blood plasma. At a pressure of 3 atm, the amount of plasma oxygen increases from 0.32 vol% (at 1 atm) to 6 vol%. This is a 95% increase in plasma oxygen concentration compared to atmospheric conditions.

The short-term effects of hyperoxia include enhanced oxygen delivery to ischemic tissues,[124] vasoconstriction, reduction of edema, and immunomodulatory properties such as activation of phagocytosis.[125,126] Long-term effects include neovascularization[122,127,128] and stimulation of collagen formation by fibroblasts.[127] HBO can thereby be applied clinically to heal hypoxic and ischemic wounds and to the recovery of radiation-injured tissue.[127,129–133]

During a standard HBO treatment, the rise in oxygen partial pressure of arterial blood can cause up to a fourfold increase in the distance that oxygen diffuses through normal tissue. There is concern that increased oxygen may stimulate tumor growth via reoxygenation of hypoxic tumor cells and increased angiogenesis as observed during wound healing. Although HBO promotes angiogenesis in healing wounds, this does not mean that it would induce tumor growth via the same mechanism.

There are several rationales for the use of HBO as an adjuvant therapy. HBO, in theory, has the potential to intercept each of the adaptations tumor cells make under hypoxic conditions (asterisks in Fig. 1). HBO greatly improves oxygen perfusion in tumors, thus altering the hypoxic microenvironment. This may have implications for angiogenesis and apoptosis and push ROS levels past the threshold level. Altering hypoxia may remove the stimulus for the angiogenic switch. HBO may promote apoptosis via the production of ROS, which can overwhelm the tumor's antioxidant defenses. Improving the oxygenation of hypoxic tumor cells may remove the hypoxic stimulus that initiates the angiogenic switch. Hypoxia is essential for stabilization of HIF-1α and subsequent VEGF expression. Reoxygenation of hypoxic cells induces rapid degradation of HIF-1α degradation[53] and subsequent VEGF production and angiogenesis in vitro.[54] ROS, at low levels, assist tumor growth but become toxic at high levels. This has been shown in vitro on human colon, liver,[106] leukemic, and ovarian[114] cell lines. HBO may increase intratumoral ROS levels past the threshold and induce tumor cell destruction, as has been shown in vitro in mouse fibroblast cells[134] and in vivo in mice with S-180 sarcoma.[135] Current opinion on the effect of HBO therapy on tumors remains controversial. Despite theoretical considerations of tumor stimulation, to date there is enough evidence to preclude any tumor stimulatory effects of HBO.

Johnson and Lauchlan first reported a tumor-stimulatory effect with HBO, demonstrating increased metastases in patients with cervical cancer.[136] This has been supported by animal studies[137–139] and other clinical trials.[136,140,141] However, in a review of animal and clinical studies conducted by Feldmeier *et al.* it was concluded that intermittent HBO exposure had no stimulatory effect on primary or metastatic tumors.[129]

In vitro studies have shown that 6 atm of absolute oxygen inhibits the growth of Erlich ascites tumor cells.[142] However, in vivo animal studies have produced varying results, with reports of both minimal and no effect.[143-147] It may be speculated that the absence of effect may be a result of tumor cell compensatory mechanisms. Such mechanisms include antioxidant defenses that would override the potential adverse effects of oxidative damage induced by ROS production during HBO treatment. Kaelin et al. showed a significant increase in the activity of SOD and improved survival of the skin flaps of rats exposed to HBO.[112] The time schedule of HBO exposure may also influence its effects. Mestrovic et al. showed significantly improved survival and reduced lung metastatic deposits in rats after HBO (3 atm) administered on days 1 to 6 or 7 to 12. However, no effect was seen in rats exposed to HBO on days 13 to 18.[148]

Experimental and clinical evidence for the effect of HBO on tumors have been varied. The data presented in the tables summarizes animal (Table 1) and clinical (Table 2) studies over the past 50 years. The outcome of each study is reported where HBO had a tumor stimulatory (+), inhibitory (–), or no (0) effect. In the literature reviewed over the past 50 years, only 10% of studies (both experimental and clinical) reported that HBO has a tumor-stimulatory effect.

Among animal studies (Table 1), there is evidence both supporting an effect with HBO and negating an effect. Studies that combined HBO with other therapies were more successful in achieving tumor control. Takiguchi et al. had more favorable results when HBO was combined with 5-fluorouracil (5-FU) than with drug treatment alone. Similarly, Petre et al. reported significantly improved tumor control with combined HBO and doxorubicin compared to drug therapy alone. It is important to note that positive effects may be tumor-specific as better tumor control was always achieved in animal studies investigating the effect of HBO on sarcomas. Huang et al. showed enhanced tumor oxygenation and improved subsequent tumor cell kill using HBO in combination with photodynamic therapy on mice with subcutaneous implantation of mammary adenocarcinoma.[16]

In studies where HBO had a tumor-inhibitory effect, a common finding in addition to overall tumor reduction was a reduction in the distribution[149] and occurrence[12,14,148,149] of distal metastases. Again, half of these studies were conducted on sarcomas. A less desirable effect was observed by Valaitis et al. on Erlich ascites tumors.[139] The remaining studies (46% of total animal studies) reported no effect with HBO. All models of human head and neck squamous cell carcinoma conducted in nude mice reported no effect with HBO therapy.

A similar trend was observed among clinical studies (Table 2) where a small number of researchers reported tumor stimulation as a consequence of HBO exposure. Most of the clinical studies investigated the use of HBO as an adjuvant to radiotherapy.

In some cases of advanced cervical[136] and bladder[140,141] cancer and one case of head and neck cancer,[150] HBO increased tumor aggressiveness when administered alongside radiotherapy. Studies incorporating a larger cohort of patients with bladder cancer did not find that HBO influenced tumor progression in combination with radiotherapy.[151–153] Patients with head and neck cancer undergoing radiotherapy were most responsive to HBO, but it improved survival in only just over 40% of cases.[151,152,154–157]

Although both animal and clinical studies have reported varied results, some deductions can be made. First, HBO does not overtly contribute to increased tumor growth, nor is it effective as a stand-alone treatment. Second, the effect of HBO is dependent on multiple factors including tumor type and stage as well as the timing, duration, atmospheric pressure, and number of HBO exposures. Regarding atmospheric pressure, whereas Dettmer et al. found no significant difference in tumor volume of transplanted Walker carcinosarcoma in rats following HBO at 1 or 3 atm,[149] Mestrovic et al. demonstrated suppression of lung metastases with improved survival in Y59 rats exposed to 3 atm but no effect at 1 atm.[148]

Among the research studies conducted in experimental models, a desirable effect was observed when HBO was combined with doxorubicin[12] and in another study with photodynamic therapy.[16] One of the mechanisms of action of doxorubicin is production of ROS. Photodynamic therapy is dependent on the presence of oxygen to destroy cells. Perhaps HBO had a positive effect in these studies because it can directly influence both ROS production and improve tumor oxygenation.

It is more difficult to draw conclusions from clinical studies due to variability in investigation techniques and patients. To date, experimental and clinical evidence of the effect HBO combined with therapies other than radiotherapy is limited. The lack of effect of HBO in experimental models as a stand-alone therapy may explain why it has not been investigated extensively in a clinical setting. Nevertheless, by altering oxygen levels in vivo, HBO can improve the radiosensitivity of tumors,[158] enhance photodynamic therapy,[16,81,159] or enhance oxidative stress and tumor cell kill of certain chemotherapy.[127,160] This has been investigated in clinical studies.

HBO AS AN ADJUVANT THERAPY

Clinically, HBO has been investigated when combined with chemotherapy, photodynamic therapy or radiotherapy. Radiotherapy induces DNA damage through the ionization of oxygen to produce ROS. Intratumoral oxygen tension therefore determine the effectiveness of radiotherapy. Hypoxia reduces the radiosensitivity of cells as they require three times as much radiation to become sensitized as cells with normal oxygen tension. HBO can be administered simultaneously with or prior to irradiation to increase the oxygen tension of hypoxic tumor cells.[155,162] Alternatively, HBO can be applied after irradiation to reduce radiation-induced tissue injury once normal tissue side effects manifest.[156,179] The objective of this is to extend the oxygen diffusion gradient to reoxygenate previously hypoxic cells and thereby radiosensitize them. It has been shown that these intercellular conditions persist for some time after leaving the chamber.

Patients are ideally irradiated prior to or while inside a pressure chamber. After numerous clinical trials this approach has been shown to be of benefit in squamous cell carcinoma (SCC) of the head and neck.[151,152,154,155,157,174,176] HBO significantly reduced

metastatic spread 3 months after irradiation of head and neck tumors and other primary tumors (breast, bowel, bladder, uterus) treated with radiation.[151] Henk et al. reported no survival difference in patients receiving radiotherapy with or without HBO. However, better local tumor control was observed in the HBO group with minimal salvage surgery compared to the non-HBO group.[11] In most of the cases, however, HBO has provided no added benefit during radiotherapy especially in the treatment of patients with cervical cancer.[169,170,172,175] Therefore, the application of HBO therapy on certain patients and tissues may be justified. However, the general consensus is that HBO does not offer any significant clinical benefits or improvement in survival. On review, a limited number of hyperbaric facilities are located in the proximity of radiation oncology departments. Although intratumoral oxygen tension persists after HBO exposure it is nevertheless temporary. To overcome this problem, HBO can be administered while patients are irradiated, but it is difficult and costly.

Clinical data obtained by the British Medical Council in a clinical trial with HBO and radiotherapy found significantly better local tumor control and survival for carcinoma of the cervix.[172] In another randomized control trial conducted in that same year, HBO therapy gave no additional therapeutic benefit and in fact increased the occurrence of late morbidity.[152] This may be due to the combined HBO group being given more fractions of radiation compared to the control group, where patients received standard fractions. This is one of the major obstacles when trying to combine HBO and radiotherapy. Generally, larger radiation doses are administered in fewer fractions to minimize the number of times patients need to enter the pressure chamber. This can lead to considerable postradiation injury to normal tissue.

A large multicenter trial conducted by Perrins et al. found no additional benefit of HBO therapy with irradiation of carcinoma of the bladder and speculated that either HBO does not alter the hypoxic state or failure of radiotherapy to cure bladder cancer is not due to hypoxic tumor cells.[78] Four other trials investigating the effect of radiotherapy and bladder cancer reported varied results, two of which suggested that combined treatment promoted tumor growth.[140,141] However, both of these trials had a small cohort of fewer than 40 patients. A meta-analysis again investigating HBO combined with radiotherapy reviewed 19 trials of tumors at various sites. Locoregional control with the combined modality was significantly greater than radiotherapy alone. The greatest effect was observed in patients with head and neck cancer.[180,181] Again, the limitations in many of these trials include the practicality of placing patients in HBO chambers while simultaneously administering radiation therapy, as reported in earlier trials.[154,155] In addition, one study reporting tumor stimulation with combined HBO/radiotherapy recruited patients with varying tumor grades.[140] Grade and stage of tumors are important determinants of treatment outcome and should therefore be evenly distributed among the various treatment groups.

In 2001, the European Society for Therapeutic Radiology and Oncology (ESTRO) concluded that the effect of HBO on neoangiogenesis and osteogenesis was graded level 1 according to evidence-based medicine criteria.[182] The COST (Cooperation in the field of Science and Technology) B14 initiative was established in 1999 to attain clinical data based on this level 1 evidence. In the most recent review of combined HBO and radiotherapy, Mayer et al. presented four randomized clinical trials that outlined the activities of the B14 working group.[13] Given the variability in past clinical trials, the four proposals presented by the COST Action B14 Committee, which have been amended and peer-reviewed, are regarded as consistent with the ''best practice'' in the field of hyperbaric medicine. At present the trials are open for enrollment of patients.

The first of four initiatives aims to determine whether HBO enhances tumor radiosensitivity in patients with previously irradiated histologically confirmed recurrent head and neck carcinoma. The second is to determine if HBO improves median survival when applied in combination with conventional fractionation in patients with glioblastoma multiforme. The last two proposals investigate the effects of HBO on postradiation injury. All aspects of the trials are kept consistent including patient recruitment, HBO regimen, radiation fractions, and outcome measures. Furthermore, it is stipulated that all irradiation fractions should precede HBO treatment, and each fraction must be given within a specified time after HBO exposure. This is the first multicenter initiative to evaluate HBO under controlled settings; and, pending results, more concrete conclusions will be possible.

Resistance to chemotherapy is common in hypoxic tumors. HBO may help overcome chemotherapy resistance by increasing both tumor perfusion and cellular sensitivity. HBO therapy in combination with chemotherapy increases cellular uptake of certain anticancer agents and the susceptibility of cells to these agents. HBO has been shown experimentally to increase the susceptibility of malignant cells to destruction with taxol,[183] doxorubicin,[12,183] and 5-FU[15,184] (Table 1).

Combined administration of 5-FU and HBO significantly increased intratumoral drug concentrations in mice implanted with sarcoma[15] and limited angiogenesis and tumor size of 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumors in rats.[184] By increasing ROS levels,[185,186] HBO enhanced the ROS-localized effects of bleomycin and doxorubicin.[20,138] In an experimental model of pulmonary sarcoma, the chemotherapeutic effects of doxorubicin were enhanced following HBO exposure at 2 atm for 7 days[12] HBO stimulated proliferation of an MCA-2 metastatic lung tumor cell line and induced cells to enter the replicating cycle compared to cells left at ambient pressure.[12] Another study found that HBO increased the percentage of prostate cancer cells in vitro accumulating in G_2/M phases from the G_0 arrest phase.[187] There was, however, only one clinical trial that evaluated HBO in combination with chemotherapy. The study reported a modest 15% improvement in local tumor control at 1 year when HBO was combined with misonidazole compared to drug therapy alone.[155] This study was conducted 20 years ago, and since then there appears to be no further clinical evidence of improved outcome of HBO with chemotherapy. Rather, HBO has been used to reduce the side effects associated with chemotherapy. Chronic arm lymphedema is a common problem in women who have undergone radiation therapy for breast cancer. HBO has been shown to reduce localized edema in a cohort of 10 women suffering from this condition.[188] Based on this evidence, patients are being recruited to determine if a more aggressive HBO regimen can further reduce the volume of edema.[189]

Photodynamic therapy (PDT) utilizes a specific wavelength of light to activate intravenously preadministered light-sensitive drugs (photosensitizing agents such as porphyrins) that are taken up by target cells. Light can then be targeted to the tumor site. Photochemical activation of the photosensitizer generates highly toxic singlet oxygen and other ROS. The response to PDT depends on adequate tumor oxygenation as well as sufficient intratumoral accumulation of the photosensitizing agent.[16,159] The effectiveness of PDT is limited by insufficient photosensitizer reaching poorly perfused tumors. HBO may improve the effects of PDT by improving both tumor perfusion and increasing the amount of singlet oxygen.

Significantly improved oxygen tension and tumor cell kill was observed with combined HBO/PDT therapy in two studies conducted on C3H mice implanted with mammary adenocarcinoma.[16,159] Promising results were found in a pilot study investigating the combined effect of HBO and PDT on patients with advanced inoperable esophageal carcinoma.[157] Tumor load in the combined PDT/HBO group was significantly lower than that observed with PDT alone. Mean survival was 12 months versus 7 months, in favor of the combined therapy.[190] Similar success was reported a year later on 30 patients, this time with inoperable non-small-cell bronchogenic carcinoma.[191] A major drawback of PDT is that administering laser therapy to the tumor site involves surgery that is more invasive than other therapies. This may explain the limited number of combined PDT/HBO trials.

Although clinical experience with HBO is generally associated with relatively few side effects, the heterogeneity of the investigation techniques makes it difficult to draw conclusions. These variations include the patients' tumor type, stage, and baseline levels. There is also variation among studies regarding the total radiation dose, number of fractions, overall time, and the irradiated volume. Furthermore, the number of trials is small with modest sample sizes (most had fewer than 200 patients). Future trials should be reported with a minimal number of variables to determine the true effect of HBO therapy. A sham therapy should be used to mask both the subjects and the assessors to HBO therapy. Employment of a double-blind trial in which patients are placed in an HBO chamber with normobaric pressure oxygen as a control compared with a chamber exposed to hyperbaric oxygen. Economic considerations should also be factored along with the practicality of the treatment in a clinical setting. Given the variation in pathology, it is not surprising that there is considerable variation in patient baseline characteristics at the time of recruitment as well as treatment outcome. Moreover, publication bias may also play a role, where results from more favorable trials may be more likely to reach completion and subsequent publication.

CONCLUSIONS

Tumors are initially susceptible to chemotherapy and radiotherapy. Advanced cancers sustain growth in the hypoxic microenvironment by adapting. ROS play a duel role in tumor growth. Initially ROS aid tumor progression via DNA damage and uncontrolled proliferation of a genomically unstable and highly aggressive cell line. In excess however, ROS are toxic to tumor cells. The effectiveness of conventional therapies is limited by the presence of hypoxia.

In theory, the use of HBO in an adjuvant setting is justified by the following: Improved oxygenation improves drug delivery to hypoxic regions in the tumor. HBO may remove the hypoxic stimulus that drives angiogenesis. Improved oxygenation may also cause

cells to enter a proliferative stage, thus sensitizing them to radiotherapy and certain chemotherapy. Increasing intratumoral ROS levels beyond the threshold may induce tumor destruction. It is apparent that the effect of HBO is dependent on the tumor's type and stage and the HBO treatment regimen. Most of the literature indicates that HBO has no impact on tumor growth—be it stimulatory or inhibitory. The most convincing effects are observed when HBO is used in an adjuvant setting, but this is specific to the tumor's type and stage. HBO therefore remains ineffective as a stand-alone therapy or even as a reliable adjuvant. Variability among investigation techniques at various centers makes it difficult to completely write off HBO as a potential therapeutic adjuvant. Further research may be warranted pending outcomes of the B14 Committee in evaluating the adjunctive potential of HBO with radiotherapy. Furthermore, consideration should be given as to the cost involved in such combined therapy against the extent of benefit that can be achieved.

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