Tumor Biology and Hyperbaric Oxygen: Differential effects in tumor versus normal tissues.

Dr. Paul Anderson
HBOT 2018 - 12th Annual International Symposium
Denver, Colorado
When assessing the potential benefits of HBOT in oncology patients occasional confusion arises when extrapolating the cytokine, epigenetic and other effects of HBOT in non-cancerous tissues (neurological and other injuries etc.) versus those in cancerous tissues.

This confusion often results in misinterpretation of data and concern over potential harm in HBOT oncology cases. The focus of this presentation is to point out the data supporting these differences as we know it to date and underscore the benefits while decreasing this clinical confusion.
Dr. Paul Anderson - Background

• CEO – Anderson Medical Group
• Chief medical advisor – Sanoviv’ Hospital
• Content Director CE site ‘ConsultDrA.com’

• Past Positions:
  • Full Research Professor – Bastyr University
  • Research Partner in NIH and other national funding sourced projects with the CUSIOS group and University of Washington, Seattle Cancer Care Alliance and Fred Hutchinson Research Center.
  • Original site director of “US Site-1” for the CUSIOS Oncology study.
Advanced Medical Therapies (Seattle, Washington)

- IV Therapies
- Mild-Moderate Hyperthermia
- Hyperbaric Oxygen
- Other specialty therapies
- Care focus is on cancer and advanced chronic illness.

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Sanoviv’ Hospital
(Rosarito, Mexico)

- Inpatient facility
- IV Therapies
- Metronomic chemotherapy
- Multi-place HBOT
- Hyperthermia
- Cancer vaccine and Rigvir programs etc. etc.
- Care for patients with Cancer and Chronic Illnesses.
HBOT 2016 & 2017

Clinical Oncology and Hyperbaric Medicine: Practice and Research Based Updates, 2017
Parts 1 & 2 – 75 Minutes

Paul S. Anderson, NMD
Advanced Medical Therapies
Seattle, Washington
Reference # 1

Targ Oncol (2012) 7:233–242
DOI 10.1007/s11523-012-0233-x

**REVIEW**

**Hyperbaric oxygen therapy and cancer—a review**

Ingrid Moen • Linda E. B. Stuhr
Reference # 2

RESEARCH ARTICLE

Oxygen-dependent regulation of tumor growth and metastasis in human breast cancer xenografts

Kristine Yttersian Sletta¹, Maria K. Tveitarås¹,²,³, Ning Lu¹, Agnete S. T. Engelsen¹,², Rolf K. Reed¹,², Annette Garmann-Johnsen¹, Linda Stuhr¹,²*

Hyperbaric oxygen as an adjunctive therapy in treatment of malignancies, including brain tumours

Katarzyna Stępień¹ · Robert P. Ostrowski¹ · Ewa Matyja¹
Li Y, Zhou C, Calvert JW, Colohan AR, Zhang JH.
Multiple effects of hyperbaric oxygen on the expression of HIF-1 alpha and apoptotic genes in a global ischemia-hypotension rat model.
Aslıcan Çakkalkurt

Hypoxia as an epigenetic factor and hyperbaric oxygen therapy.

J Clin Epigenet 2017, 3:4 DOI: 10.21767/2472-1158-C1-002
Reference # 14

Jung et al.

The impact of hyperbaric oxygen therapy on serological values of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF).

Head & Face Medicine 2010, 6:29 http://www.head-face-med.com/content/6/1/29
Reference # 17

Bremnes, Roy M. et al.
The Role of Tumor Stroma in Cancer Progression and Prognosis: Emphasis on Carcinoma-Associated Fibroblasts and Non-small Cell Lung Cancer
Journal of Thoracic Oncology, Volume 6, Issue 1, 209 - 217
Janine M. DeBlasi, Nathan P. Ward, Angela M. Poff, Andrew P. Koutnik, Christopher Q. Rogers, and Dominic P. D’Agostino

Anti-Cancer Effects of Ascorbic Acid and Hyperbaric Oxygen Therapy in vitro

The FASEB Journal 2017 31:1_supplement, 879.4-879.4
Targeting Cancer Metabolism with Ketosis and Hyperbaric Oxygen

January 2014

Angela M. Poff
University of South Florida, abennett@health.usf.edu
Other Resources not directly referenced:

Paul G. Harch, MD
Oxygen and Pressure Epigenetics: Understanding Hyperbaric Oxygen Therapy After 355 Years as the Oldest Gene Therapy Known to Man
Townsend Letter; Page 30 - Tuesday, 13 March 2018
Other Resources not directly referenced:

**Hyperbaric oxygen therapy for cancer treatment side effects.**

<table>
<thead>
<tr>
<th>Subject:</th>
<th>Hyperbaric oxygenation (Complications and side effects) Cancer (Care and treatment) Cancer (Methods) Cancer (Patient outcomes)</th>
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<tbody>
<tr>
<td>Author:</td>
<td>Faass, Nancy</td>
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<td>Pub Date:</td>
<td>08/01/2012</td>
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<tr>
<td>Publication:</td>
<td>Name: Townsend Letter Publisher: The Townsend Letter Group Audience: General; Professional Format: Magazine/Journal Subject: Health Copyright: COPYRIGHT 2012 The Townsend Letter Group ISSN: 1940-5464</td>
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<td>Issue:</td>
<td>Date: August-Sept, 2012 Source Issue: 349-350</td>
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Key Point:

Normal tissue and wounded tissue DO NOT act like Tumor and Tumor Environments under HBOT

HBOT Effect in one ≠ The other!
Fig. 1 Hypoxia is a hallmark of solid tumors. Summary of the hypoxia-induced factors influencing cancer growth and progression.
Epithelial-mesenchymal Transition (EMT)
Type 3 EMTs occur in the context of tumor growth and cancer progression, when cancer cells at the invasive front of the tumors convert to a mesenchymal phenotype.

The induction of type 3 EMTs is facilitated by the genomic alterations acquired by cancer cells and these EMTs generate cells with invasive properties that enable them to move into the blood stream and spread systemically to other organs.
Type 3 EMT

Primary epithelial cancer cell

Invasive and metastatic cell
The effect of HBOT on Epithelial to Mesenchymal Transition (EMT) signalling

EMT has been indicated to play an important role in the development of cancer and metastasis and thus the specific markers E-Cadherin and N-Cadherin were investigated using western blot. Immunoblots from MDA-MB-231 tumor lysates indicated a downregulated N-cadherin expression in HBOT tumors. The downregulated N-cadherin in HBOT tumors was confirmed i.e. “via the N-cadherin pathway (see below) under HBOT therapy (2.47 ATA in Mice) EMT was down-regulated.
EMT Post-HBOT (less N-cad and AXL) [2]

Fig 6. Epithelial to mesenchymal transition after hyperbaric oxygen treatment. MDA-MB-231 tumors constitutively express E-cadherin (A) and N-cadherin (C) in western blot lysates in both groups. The expression of E-cadherin (B) and N-cadherin (D) when adjusted according to loading control (β-Actin) in control (n = 5 tumors) and HBOT (n = 5 tumors) is also shown. Mean ± SD. *p>0.05. Immunofluorescent staining of cryosections from HBOT and control MDA-MB-231 tumors confirm the reduced nuclear N-cadherin and also reveals a reduction in the expression of receptor tyrosine kinase Axl (E). Scalebar: 25μm.
Of note: MET in any paper or figure is EMT reversing.
Hypoxia Induction Factor

HIF-1
HIF-1 and HBOT in NORMAL Tissue

https://openi.nlm.nih.gov/imgs/512/203/4292106/PMC4292106_IJPS-47-303-g001.png

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HIF-1 and Tumors

Hypoxia, via HIF-1a, causes translocation to the nucleus and increase the transcription of multiple genes associated with this aggressive behaviour. Thus, since HBOT enhanced the pO2 in tissues temporarily, and O2 is important for degrading HIF-1a, we expected reduction in tumor growth. [2]
HIF-1-mediated transcription

Angiogenesis
- VEGF
- Endoglin
- Leptin
- TGF-β3
- PDGF-β
- NOS

Metabolic Adaptation
- Enolase
- Hexokinase 1,2
- GLUT-1
- GADPH

Survival/Proliferation
- EPO
- IGF2
- TGF-α
- Cyclin G2

Invasion
- MET
- MMP-2
- AMF
- UPAR
- Cathepsin

HIF-1 implicated in every aspect of cancer progression

Regulation of cancer cell metabolism by hypoxia-inducible factor 1; Semenza, G.

30-60 mmHg  
2-30 mmHg  
Average PO₂

Confers chemo/radioresistance
In non-cancerous inflamed tissue HBOT does not generally lower HIF-1 [5]

OBJECTIVES: This study investigated the role of hypoxia-inducible factor 1alpha (HIF-1alpha) in acute pancreatitis (AP) and whether HIF-1alpha is involved in the therapeutic effects of hyperbaric oxygen (HBO) on AP.

METHODS: Thirty Wistar rats with taurocholate-induced AP were randomly assigned to 3 groups...

RESULTS: The HBO therapy attenuated the severity of acute pancreatitis; ... The HBO therapy inhibited AP-induced up-regulation of HIF-1alpha and its downstream effector [VEGF] and the production of [TNF]-alpha and myeloperoxidase activity.

CONCLUSIONS: Hypoxia-inducible factor 1alpha plays a key role in the pathogenesis of AP, and the ability to down-regulate the expression of HIF-1alpha may partially explain the therapeutic effect of HBO on AP.
HIF-1 and VEGF

HIF-1α is a key oxygen-regulator of VEGF gene expression which is important for angiogenesis and thus tumor growth. [2] → REDUCING TUMOR HYPOXIA DECREASES VEGF.

http://journals.plos.org/ploscompbiol/article/figure/image?download&size=large&id=info:doi/10.1371/journal.pcbi.1004612.g001
In vitro no significant vascular diameter change and vessel density rose in 1 cell type at 2.5 BAR (2.47 ATA) in cell culture over 24 days. [2]

Table 1. Blood vessels.

<table>
<thead>
<tr>
<th></th>
<th>MDA-MB-231</th>
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<th>BT-474</th>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>HBO</td>
<td>Controls</td>
<td>HBO</td>
</tr>
<tr>
<td>Pressure pO2</td>
<td>1 bar</td>
<td>2.5 bar</td>
<td>1 bar</td>
<td>2.5 bar</td>
</tr>
<tr>
<td>% O2</td>
<td>20</td>
<td>100</td>
<td>20</td>
<td>100</td>
</tr>
<tr>
<td>Blood vessels density (number/mm²)</td>
<td>13.5 ± 1.6</td>
<td>12.4 ± 1.8</td>
<td>11.1 ± 2.2</td>
<td>13.1 ± 4.5</td>
</tr>
<tr>
<td>Blood vessel diameter (μm)</td>
<td>7.3 ± 1.7</td>
<td>7.2 ± 1.8</td>
<td>12.0 ± 6.1</td>
<td>12.3 ± 6.4</td>
</tr>
</tbody>
</table>

Blood vessel density and diameter in MDA-MB-231 and BT-474 tumors in control and after hyperbaric oxygen treatment for 24 days. Means ± SD.
Aggravation of Macular Degeneration

HBOT in NORMAL TISSUE does not generally lower VEGF [16]

Background: Hyperbaric oxygen (HBO) therapy is an effective adjunct treatment for ischemic disorders such as chronic infection or chronic wounds. It combines hyperoxic effects with the stimulating potential of posttherapeutic reactive hypoxia. As its crucial effects, stimulation of fibroblast growth, induction of collagen synthesis and the initiation of angiogenesis are discussed. Angiogenesis is a multistage process resulting in the growth of blood vessels. It includes degradation of extracellular matrix, proliferation and migration of different cell populations and finally formation of new vessel structures. This complex chain of procedures is orchestrated by different cytokines and growth factors. Crucial mediators of angiogenesis are basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF); their in-vivo function is still not fully understood.
HBOT in NORMAL TISSUE does not generally lower VEGF [16]

Methods: Forty-three patients suffering from sudden sensorineural hearing loss or tinnitus were treated with HBO. The therapy included 10 sessions of 90 minutes each, one session a day. [* 1.55 BAR / 1.53 ATA] Serological levels of bFGF and VEGF were assessed by [ELISA] on day 1, 2, 5 and 10 of HBO therapy and were compared to mean values of the control group, related to the patient’s age and sex, and their development observed over the ten days of HBO.

Conclusions: A significant effect of HBO on serum concentrations of bFGF and VEGF was not verified in the present study. Additional application of exogenous growth factors in conjunction with HBO was not obviously linked by a coherent cause-and-effect chain as far as wound healing is concerned.
In non-cancerous inflamed tissue HBOT NORMALIZES VEGF and decreases TNFa... [5]

OBJECTIVES: This study investigated the role of hypoxia-inducible factor 1alpha (HIF-1alpha) in acute pancreatitis (AP) and whether HIF-1alpha is involved in the therapeutic effects of hyperbaric oxygen (HBO) on AP.

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Apoptosis
Apoptosis

• Apoptosis (programmed cell death) is one of those processes that are only supposed to go one way.
  • Once begun, there is no turning back.

• The cell begins to degrade itself through the activation of caspases, proteolytic enzymes that cleave the structural elements of the cell, leading to a series of morphological and biochemical changes.
  • The cell digests itself from the inside, turning itself to a colloid of dead organic molecules inside the cell membrane.
  • In the end, it fragments neatly into membrane-enclosed vacuoles that are then phagocytosed by nearby cells.
Apoptosis Induction Factor (AIF) processing is sequentially regulated by Ca2+ and ROS.
• Studies of apoptosis in neoplasms treated with HBO are limited. **Two in vitro studies on mammary and oral cancer cells**, respectively, showed **no change** in apoptosis after HBO [18, 19].

• On the other hand, Chen et al. [20] **observed activation of the pro-apoptotic pathway MAPK and downregulation of the anti-apoptotic ERK pathway** in hematopoietic cells after HBO.

• Additionally, a study of HBO using osteosarcoma cells also demonstrated **induction of apoptosis** [21].

• In two different **animal models**, gliomas and mammary tumors, respectively, our group has demonstrated **induction of cell death after HBO treatment** [22–24].
• Furthermore, reduced cell proliferation, together with a significant change in histology, has also been shown after HBO treatment in DMBA-induced mammary tumors in vivo [22, 24].

• Granowitz et al. [18] observed the same reduction in cell proliferation in their mammary in vitro study. In addition, two recent studies on osteosarcoma cells [21] and nasopharyngeal carcinoma [25] support inhibition of cell division after HBO treatment.
Hypoxic cancer cells survive via the adaptation to the unfavorable conditions. Moreover, they are related with tumor recurrences and interfere with treatment success in many types of malignancies.

Hypoxia affects angiogenesis, apoptosis and many gene products that are involved in glycolysis directly.
The **target genes regulated** by hypoxia mediate tumor progression **through an epigenetic mechanism**. **Hypoxia** is essential for hypoxia-inducible factor (HIF) \(-1\alpha\) stabilization and following vascular endothelial growth factor (VEGF) expression.

**Tumor oxygenation can be increased via hyperbaric oxygen therapy (HBOT).**
Hypoxia, Apoptosis and HBOT [6]

HBOT increases oxygen perfusion of the tumor tissues, changes hypoxic microenvironment, can increase apoptosis via ROS production and abolish antioxidant defense mechanisms of the tumors.
In non-cancerous brain tissue HBOT decreases apoptosis during ischemia-reperfusion. [4]

“... These results indicate that HBO has multiple actions on apoptotic genes even though the overall effect of HBO was decreased HIF-1alpha expression and reduced apoptosis after global ischemia-hypotension.”
http://www.mdpi.com/cancers/cancers-08-00103/article_deploy/html/images/cancers-08-00103-g002.png
Hyperactivated mTOR pathway leads to dysregulation of translational control and cancer.
We also demonstrated a reduction in the expression of Axl RTK in the HBOT tumors. Axl RTK has been correlated with poor outcome and drug resistance in a wide range of cancer types, and has been shown to be necessary for maintaining tumor EMT. Axl RTK has been shown to be upregulated in human breast cancer samples, and to be associated with a reduced patient survival.

Furthermore, Axl RTK expression remained an independent negative prognostic factor in a multivariate analysis including basic prognostic factors, and a hazard ratio of 3.27 were detected between cases with high and low Axl expression. [2]
In an experimental tumor model, the authors demonstrated that Axl knockdown completely prevented the spread of highly metastatic breast carcinoma cells from the mammary gland to lymph nodes and several major organs and increased overall survival.

Taken together, it is likely to assume based on the findings from our study that enhanced oxygen could be critically involved in MET transition of developing breast tumors, and also be implicated in the reduced metastatic potential observed after HBOT in the present study. [2]
N-cadherin
And although nuclear localisation of N-cadherin has rarely been described in human solid tumors, it has been shown to correlate with poor prognosis in nasopharyngeal carcinoma (NPC). In this study, a high expression of nuclear N-cadherin predicted a poorer survival in patients with late stage disease, and multivariate analysis showed nuclear N-cadherin to be an independent prognostic marker for NPC patients. [2]
In the present MDA-MB-231 model, we found a significant reduction in N-cadherin after HBOT. In most types of cancer, high N-cadherin expression correlates with cell motility, invasion and metastasis. Furthermore, N-cadherin was demonstrated to be localized to the nucleus in our experimental tumor model, and aberrant N-cadherin in the nucleus has been shown to be necessary for cell migration during EMT. [2]
Tumor Stroma
Tumor Stroma (depending on signaling) Promotes Cancer Progression [17]

Maintenance of both normal epithelial tissues and their malignant counterparts is supported by the host tissue stroma. The tumor stroma mainly consists of the basement membrane, fibroblasts, extracellular matrix, immune cells, and vasculature. Although most host cells in the stroma possess certain tumor-suppressing abilities, the stroma will change during malignancy and eventually promote growth, invasion, and metastasis. … During recent years, the crosstalk between the cancer cells and the tumor stroma, highly responsible for the progression of tumors and their metastasis, has been increasingly unveiled. …
Normal epithelium → Malignant transformation → Cancer

Normal stroma: Fibroblasts, immune cells, endothelial cells, ECM

Cancer stroma: Fibroblasts↑, immune cells↑, endothelial cells↑, ECM↑

Tissue integrity

Invasion
Effects of HBOT on collagen content and proportion of stroma versus tumor cells

Analysis of immunofluorescent stained collagen type I fibrils in primary tumors, using Image J, revealed significant (p<0.001) reduced density in HBOT compared to controls as shown in Fig.7, panels A, C-D. Analysis of immunofluorescent stained Itgb1 did not show any differences between controls and HBOT (B), neither did FSP1 as shown in S1 Fig. The amount of stroma versus tumor cells are also demonstrated in Fig.7, panels E-F, demonstrating significantly less cancer cells in the HBOT group compared to control.
SUMMARY POINTS
• **Original** in-vivo study showing **increase in cancer** with HBOT (head-neck in mouse) 2.4 ATA @ 90 min, 7 days/week X +/- 30 dives. [11] – This and a few other studies have caused the “HBOT promotes cancer concerns” widely reported.

• Numerous papers noted above and excellent reviews of the topic in 2012 and 2016 [1, 3] show that **these concerns are unfounded**.

• **Tumor response to HBOT** (and tumor biology) are not analogous to normal or non-cancerous injured tissue biology or pathology.
• Globally speaking lower O2 in the tumor environment ENHANCES tumor growth and metastasis as an adaptive mechanism. [1]

• HBOT decreases tumor cell survival, metastasis, angiogenesis and other key promoting factors. [1, 2, 12, 13, 14]

• HBOT has been shown to be associated with increased survival in humans with cancer. [3]

• HBOT alters gene expression to switch to a lesser tumorigenic metabolism [1]
• Conventional and experimental oncology therapies shown to have potential synergy with HBOT include (but are not limited to):
  • General Chemotherapy Uptake Improvement [1,3]
  • 5-FU [1,3]
  • Platinums [1]
  • Taxanes [1]
  • Radiation [1,3]
  • Artemisinin / Artesunate [1]
  • TMZ (Temozolomide ) [3]
  • Ascorbic Acid [18,19,20]
Important Therapy-Synergy Note:

• With oxidative therapies always start with lower pressures (1.3 to 1.5 ATA) and do a test dive at 45 minutes bottom time.

• This is especially critical if HBOT and the oxidant therapy are administered on the same day.

• For other synergy “lessons learned” see my next presentation.
Thank you!