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Hyperbaric Oxygen, Stem Cells

&

The Spine Interventionalist

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I wish to thank Claire Tibiletti, M.D. for inviting me here to share my excitement about Hyperbaric Oxygen and it’s potential benefit for patient outcomes in your interventional spine practice.
It has been established that hyperbarics has an effect on stem cell release and of course hyperbarics has an extensive history in wound care protocols. Radiation necrosis, carbon monoxide poisoning, etc. and is approved in 13 different medical indications.

Simple Gas Law Physics (Boyle’s, Henry’s, Dalton’s, etc.) indicate how O₂ is pushed into fluids of the body beyond the hemoglobin on the red blood cell. Oxyhemoglobin will pass unchanged from the arterial to the venous side because O₂ physically dissolved in solution will be utilized more readily than that bound to hemoglobin.
A number of studies suggest hyperbaric oxygen will mobilize stem cells in the body making them available for repair. This appears to increase nitric oxide production which directly increases stem cell production and release.

A study by Stephen Thom, et al. (University of Pennsylvania) showed that hyperbaric oxygen will cause rapid mobilization of stem/progenitor cells in humans. The mobilization is thought to be caused by a nitric oxide (NO) dependant mechanism. Over a course of 20 treatments, the CD34+ cells increased eight fold (800%).
The population of CD34+ cells in the peripheral circulation of humans doubled in response to a single exposure to 2 atmospheres absolute (ATA) O₂ for 2 hours. Over a course of 20 treatments, circulating CD34+ cells increased eightfold.”


This is due to NO reaction at endothelium changing the artery wall so that a Diabetic patient can release their own stem cells into circulation.
Lotovin, et al. (1981) reported a study of the problem of cellular and hormonal activity under hyperoxia. They found six sessions of HBOT @ 2.5 ATA resulted in an increase of T-Lymphocytes in guinea pigs. When pressure was raised to 5 ATA for 30 min, a form of oxygen toxicity occurred with depression of functional activity of T-Lymphocytes and a decrease in cellular indices of immunity in the blood. The animals recovered 10 days after this exposure.

In patients given 15 daily sessions of HBO at 2.5 ATA (60 min), the number of T-Lymphocytes increased 1.4 fold and that of B-Lymphocytes 2.8 fold. There was also an increase in all the immunoglobulins. Biriukov et al (1988) observed the number of lymphocytes is diminished after surgery and that HBO stimulates lymphocyte production and improves the patients resistance against infections in the post operative period.
Effects of hyperoxia on neutrophil adhesion.

S.R. Thom - Institute for Environmental Medicine and Department of Emergency Medicine University of Pennsylvania Medical Center. Philadelphia, PA

“Based on a rather wide sampling of disease processes, inhibition of neutrophil attachment to blood vessel appears to be a common theme to the beneficial effects of hyperbaric oxygen”
“Adequate oxygen tension is a prerequisite for the formation of collagen matrix, which is essential for angiogenesis. In irradiated tissue, hyperbaric oxygen is more effective than normobaric oxygen in increasing the partial pressure of oxygen to a level that promotes the formation of collagen matrix and angiogenesis.

Reperfusion injury can worsen crush injuries and compartment syndromes and cause skin flaps and reattachment procedures to fail. Neutrophils have been implicated as the prime endogenous culprit in reperfusion injury. Adhering to the walls of ischemic vessels, they release proteases and produce free radicals, leading to pathologic vasoconstriction and extensive tissue destruction. Hyperbaric oxygen inhibits neutrophil adherence and post-ischemic vasoconstriction in ischemic rat tissue.” 26 Patrick M. Tibbles, MD Hyperbaric Oxygen Therapy. The New England Journal of Medicine. June 20,1996. 1642
Hypoxia Inducible Factor – 1 alpha subunit, also known as HIF-1α

This protein is found in mammalian cells growing at low oxygen concentrations. It plays an essential role in cellular and systemic responses to hypoxia. 33
“Hypoxia causes macrophages, neutrophils and other cells to activate a variety of their genes, including those encoding vascular endothelial growth factor (VEGF), which makes blood vessels leaky. This gene activation is achieved by means of HIF-1α, which binds to the promoters (regulatory elements) of target genes. To do so, HIF-1α must first bind its partner, HIF-1β, and this interaction is controlled by oxygen levels. When oxygen is abundant, there is little HIF-1α – it is destroyed under the direction of the von Hippel-Lindau (VHL) protein – and what there is can’t bind HIF-1β. At low oxygen levels these twin restraints are lifted. 21

Blue = Prolyl hydroxylases enzymes inhibited by hypoxia that otherwise restrict HIF-1α.

Purple = Cramer, et al. HIF-1α redness, swelling from glycolytic enzymes.

Green = Increased nitric oxide increasing HIF-1α with reduced ATP synthesis.
“The major discovery about the use of oxygen has been it’s role through Hypoxia Inducible Factor 1Alpha (HIF-1α) in gene control.”  

20 Phillip James, MD, ChB, DIH, PhD, FFOM
Emeritus Professor of Medicine, University of Dundee, Scotland.

“The protein, Hypoxia Inducible Factor 1Alpha (HIF –1α), regulates the expression of at least 30 genes when oxygen levels are low. Cramer et al. shows that HIF-1α also controls several key aspects of inflammation: the redness and swelling of injured tissues, and the ability of leukocytes to enter these sites. It is striking that a single molecule should emerge as a master regulator in two such diverse and significant settings as hypoxia and inflammation.”

21 Carl Nathan Department of Microbiology and Immunology Weil Medical College, Cornell University. Oxygen and the inflammatory cell

Hypoxia and Inflammation
Holger K. Eltzschig, MD, PhD and Peter Carmeliet, MD, PhD
February 17, 2011
We discuss the regulation of immune responses by hypoxia-induced signaling, outline molecular aspects of the cross-talk between hypoxia and inflammation, and illustrate the link between hypoxia and inflammation in inflammatory bowel disease, certain cancers, and infections.
Figure 1. Links between Hypoxia and Inflammation.
Shown is an overview of clinical conditions characterized primarily by tissue hypoxia that causes inflammatory changes (left) and inflammatory diseases leading to tissue hypoxia (right).
We stress that in the case of inflamed tissue, hypoxia is not a bystander but instead can influence the environment of the tissue, particularly by regulating oxygen-dependent gene expression.\textsuperscript{31}
Hypoxia suppresses the response of the adaptive immune system.
Concentrations of oxygen in solid tumors, as compared with those in normal tissues, are frequently reduced. Solid tumors contain increased levels of HIF-1α and HIF-2α, and these elevated levels correlate with cancer-related death.  

In breast cancer, the mean PO2 is 10 mm Hg (as compared with >60 mm Hg in normal breast tissue), and a PO2 of less than 10 mm Hg in the primary tumor is associated with increased risks of metastasis and death.
HIF-1α inducing transcription of numerous genes, including inflammatory genes.
Experimental evidence indicates that inhibition of HIF within the inflamed tumor core attenuates the growth and vascularization of tumors and enhances the sensitivity of tumors to radiation. When oxygen is abundant, there is little HIF-1α – it is destroyed under the direction of the von Hippel-Lindau (VHL) protein – and what there is can’t bind HIF-1β. At low oxygen levels these twin restraints are lifted.
Cellular Therapies

One goal is to replace or restore tissue’s reparative functions that have been lost because of injury, the aging process, or disease. Transplanted cells can act as pharmacological agents, exerting paracrine influences or stimulating the cytokine secretion of donor cells to modulate the host’s regenerative response. Transplanted donor cells themselves may regenerate host tissue.
Skeletal muscle stem cell mediated regeneration

Fabrisia Ambrosio, PhD, MPT
Cellular Rehabilitation Laboratory of the Stem Cell Research Center
Departments of Physical Medicine & Rehabilitation, Physical Therapy and Orthopedic Surgery
University of Pittsburgh
• Muscle declines start as early as the mid-twenties!

• Increasing age is commonly associated with skeletal muscle regenerative declines, which is associated with impaired mobility and increased likelihood for falls.
Microenvironment (niche)

Young Muscle:

Myofiber

Old Muscle:

Muscle stem cell
Can rehab modalities be used to enhance the intrinsic functional capacity of muscle stem cells?
Aged skeletal muscle serves as an example. Aged muscle is characterized by a significant decrease in both the local secretion of critical growth factor (VEGF) \(^{10,11}\) and the number of cells responsible for muscle regeneration (muscle stem cells).\(^{12,13}\) Together, these deficits result in considerably diminished healing potential of aged muscle.

Cell based strategies hold promise. Advances in stem cell biology have determined that there are populations of stem cells that persist throughout adult life. \(^{14,15,16}\) Such cells eliminate ethical concerns about embryonic cells.
There is substantial evidence that exercise, mechanical stimulation or both play a critical role in the success of neurogenerative and musculo-skeletal regenerative therapies.

Mechanical stimulation modulates the cellular niche and the use of forces can help the donor cells integrate into the body in a useful and functional way.

Traditionally, stem cell transplantation has been to introduce the cells and hope for the best response. Targeted mechanical stimulation provides a means for communication with the cells after transplantation.
Both in vitro and in vivo, stem cells are amenable to modulation by external mechanical forces.\textsuperscript{17}

Success of Orthopedic surgery is largely reliant on effective rehabilitation programs after surgery. The need to implement targeted rehabilitation programs soon after stem cell transplantation to maximize functional outcomes is clear.

• Electrical stimulation for two weeks increases vascularity.\textsuperscript{32}

• Electrical stimulation results in improved myofiber regeneration at both 5 and 10 days following injury.

• Electrical stimulation decreases fibrosis at 5 and 10 days.

• Electrical stimulation doubles contractive force of muscle in 4 weeks.
Fibrosis is inversely proportional to skeletal muscle vascularity. Hyperbaric Oxygen increases vascularity as previously mentioned and increases CD34+ stem cells by a factor of up to 8 (800%) and the oxygen concentration in tissue up to 2,000%. 19
Photo Modulation

Photo modulation seems to work on both PRP components and stem cells. Stem cells respond to low level lasers

1. PRP plus Autologous conditioned serum
2. “healing and anti-inflammatory”
3. Growth factors from platelets (healing)
4. IL1ra from WBCs (potent anti-inflammatory)
5. Beta-endorphin from WBCs (pain relieving)
6. Pro-inflammatory cytokine receptor shedding from WBCs (anti-inflammatory)
7. Similar to German process called “Orthokine”
Bioscaffolds or Extracellular Matrix for Orthopedic applications?
Dr. Stephen Badylak, DVM, PhD, MD et al. designed a tissue engineering construct comprising the same structure as the body’s naturally occurring scaffold material, extracellular matrix (ECM).

In the case of a large tendon defect, the ECM provides a supportive medium for vessel and collagen growth and degradation of the scaffold over time triggers the formation of natural tissue in it’s place. 1,2,3.
The time course of tendon regeneration events after the application of an ECM scaffold obtained from small intestinal submucusa has been well characterized and is illustrated in the figure below:

Figure.
Demonstration of tendon gap injury repair after the application of a small intestine submucosal (SIS) extracellular matrix scaffold. bFGF = basic fibroblast growth factor, TGF-β1 = transforming growth factor β1, VEGF = vascular endothelial growth factor. Based on Gilbert et al. 6
The presence of donor ECM also appears to promote the infiltration of progenitor cells and bone marrow stem cells into the site of injury, a phenomenon associated with an enhanced regenerative response after injury.
Given that revascularization of the treated area is critical to promote tissue healing, scaffolds offer the further advantage of possessing angiogenetic properties \(^4,5\) which are also related to mechanical loading.\(^6\)

Targeted rehabilitation modalities, such as, a chronic, low intensity electrical stimulation protocol designed to target therapeutic angiogenesis \(^7,8,9\) works synergistically with an ECM scaffold to further enhance treatment efficacy. \(^8\)
Turns out….

- Mechanical stimulation is critical for scaffold remodeling as well…
  
  A. Electrical stimulation.
  
  B. Exercise.
  
  C. Hyperbaric Oxygen increases vascularity.
“Hyperbaric Oxygen is known to create angiogenesis and may enhance the microenvironment where revascularization is considered critical to promote true healing.” 18
Implantable materials for regenerative medicine: We are designing a new family of biomaterials that are made entirely of engineered proteins. By carefully selecting the primary amino acid sequence of our engineered proteins, we can create biomaterials with independently tunable biochemical and biomechanical properties that mimic many of the essential properties of natural tissues including elasticity, proteolytic remodeling, and cell binding and signaling. An essential component of these engineered protein-based materials are elastin-like peptide sequences that provide excellent mechanical resilience. These elastin-like biomaterials are being investigated for use both as ex vivo tissue mimics to study the fundamentals of cell-matrix interactions and as in vivo tissue mimics for regenerative medicine applications. Current systems under study include neuronal, cardiac, vascular, and bone tissues amongst others.
Stem Cells

“Patients and surgeons prefer injecting through a syringe needle, because this is the least invasive way. However, the injection process often damages many of the cells. By encapsulating the cells in a protective biomaterial gel, my group has found that we can greatly improve the efficiency of transplantation.” — Sarah Heilshorn, Asst. Professor Stanford University. The Brain – A Discover Magazine Special. Spring, 2012.
Biomaterial Design Strategies for the Treatment of Spinal Cord Injuries

“A comprehensive review of biomaterial-scaffold design strategies currently being applied to the development of nerve guidance channels and hydrogels that more effectively stimulate spinal cord tissue regeneration.” 23 Sarah Heilshorn, et al Journal of Neurotrauma 27:1-19 January, 2010
Chronic Pain?

“An astonishing transition in medical science is a painkiller that does not act on pain neurons; it brings relief from chronic pain by acting on nonnueronal cells.” 24

“Minocycline acts on microglia that release fractalkine when nerve cells are damaged preventing the microglia release of inflammatory and painful cytokines.” 24

“Minocycline attenuates T cell and microglia activity to impair cytokine production in T cell-microglia interaction” 29

“While most other HBO pain studies focus on inflammatory mechanisms, we believe that HBO also reduces pain by acting in the brain. Studying the mechanism of how HBO can reduce neuropathic pain can reveal molecular targets in the brain and possibly stimulate the development of new drugs that act on the same targets for long-term relief of chronic pain.” 27

“Both models demonstrated significant improvement in response to treatment over the course of the two-week period, with chronic constriction injury (CCI) animals recovering more quickly and maintaining this recovery throughout the post-treatment period. Hyperbaric oxygen treatment appears to be successful in relieving neuropathic pain for an extended period of time.” 28
The “take home message” is that Hyperbaric Oxygen is a systemic treatment with little to no risks and *when oxygen is abundant*, there is little hypoxia inducible factor – HIF -1α and Neutrophil attachment is inhibited in vessel walls.
Thank you!

Ray Crallé, R.P.T.

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