HYPERBARIC OXYGENATION: WOUND TREATMENT

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This review discusses different issues of hyperbaric oxygen therapy (HBOT) on wound treatment.

**Basis.** The clinical use of HBOT consists in breathing oxygen (O₂) at 100% in a pressurized chamber, of at least at 1.4 absolute atmospheres (ATA). Under these conditions, a large amount of O₂ is dissolved in the plasma and promptly used by all cells, reaching poorly perfused tissues.

**Biochemical events.** HBOT acts producing both hyperoxia and reactive oxygen species (ROS) and stimulating the activity of antioxidant systems. Hyperoxia triggers biochemical mechanisms, some of them, vasoconstriction, angiogenesis, anti-inflammation, fibroblast stimulation and cell mediated immune response, stand out as benefits in wound treatment. Such biochemical markers are used to track beneficial events from HBOT, as they can vary by its therapeutic action.

**Applications.** Several indications of this therapy in various diseases are widespread and in continuous research and development. Literature is plenty of available scientific papers and protocols reporting HBOT uses in different specialties, including the clinical area, sport medicine, orthopedics, neurology and wounds recovery. In problem wounds, TOHB is used as adjuvant treatment, performing its therapeutical effect through hypoxia reversion, improvement in vascularization and healing acceleration.

Keywords: Hyperbaric Oxygenation, Chamber, Biomarkers, Wounds
Acronyms:
ATA: absolute atmospheres
EPO: eritropoyetin
FR: free radicals
Hb: hemoglobin
HBO: hyperbaric oxygen
HBOT: hyperbaric oxygen therapy
HIF: hypoxia inducible factor
NOS: nitric oxide synthase
O₂: oxygen
Pp: partial pressure
PpO₂: oxygen partial pressure
PtcO₂: transcutaneous oxygen tension
ROS: oxygen reactive species
VEGF: vascular endotelial growth factor
1. **Hyperbaric oxygen therapy: basis and oxygen physiology**

HBOT consists in breathing $O_2$ near to 100% within a pressurized chamber above the normal atmospheric pressure (at sea level, or 1.0ATA). For clinical use, the pressure should be at least 1.4ATA \[1\]. Hyperbaric oxygenation (HBO) is used as a primary therapy \[2\], in certain diseases and intoxications, or as an adjunctive therapy in pathologies with inadequate oxygen supply to the tissues.

**Physiology of oxygen**

Hyperbaric chambers are medical devices where HBOT is performed in a non-invasive and safe fashion: high $O_2$ concentration is administered to the patient by means of a mask, within a pressurized environment. In order to understand how this therapy works, it is important to keep in mind the main function of the breathing process: oxygen enters the body, to be distributed throughout the circulatory system to all organs and tissues.

**Physical basis**

The physical-chemical basis of the therapy is essentially based on two physical laws that describe gas behavior. On one hand, Dalton's Law states that, at constant temperature, the total pressure of a gas mixture is equal to the addition of partial pressures (Pp) of each individual gas. In other words, each gas exerts a pressure proportional to its fraction in the total volume of the mixture \[3\]. Therefore, when using roughly 100%$O_2$ at 1.4ATA pressure, high Pp$O_2$ is obtained, several times greater than in normal conditions (breathing normal air: 21%$O_2$, 1.0ATA). On the other hand, Henry's Law states that gases are dissolved in liquids when they are subjected to pressure: meaning that administered $O_2$ in a pressurized environment, is dissolved and distributed in the plasma and other fluids in contact with gas \[3\]. This effect takes place once the amount of inspired $O_2$ increases, generating a local pressure gradient in the alveoli, favoring the diffusion of oxygen into the plasma. Moreover, this mechanism is
independent from the transport of \( \text{O}_2 \) bound to hemoglobin (Hb), which is almost completely saturated (~97%) under physiological conditions [3].

The purpose of HBOT is to assure that \( \text{O}_2 \) enters the tissues, without the contribution of \( \text{O}_2 \) from Hb, in cases of obstruction of red blood cells flow (edema, inflammation) and in anemic patients [3]. Thus, most \( \text{O}_2 \) is dissolved in the plasma and a high concentration of circulating \( \text{O}_2 \) turns available to diffuse, reach and penetrate into tissues and cells.

**Physiological basis**

Once understood the diffusive behavior of \( \text{O}_2 \) in plasma, it is important to understand, through a model, how tissues and their cells receive \( \text{O}_2 \) during HBOT. The answer follows the Krogh model [4], which considers capillary density in tissues, capillary radius and the distance between cells and capillaries to calculate the \( \text{O}_2 \) diffusion distance and penetration. For example, depending on their function and metabolic rate, different organs and tissues of the organism have different density of blood vessels (capillaries and arterioles) per volume (100 to 3000 vessels/mm³) [4]. In addition, Krogh’s model explains the existence of radial and longitudinal pressure gradients (PpO₂), depending on the radius of the capillary and the arterial and venous ends of the microvasculature, respectively (see figure 1). From the combination of these variables, the model allows prediction of PpO₂ in tissues: when \( \text{O}_2 \) is administered at a concentration near to 100% in a 1.4ATA environment, the \( \text{O}_2 \) penetration radius from capillaries to tissues is ~75µm.
Effective hyperbaria

It is important to remind the concept of effective hyperbaric and the clinical use definition for HBOT [1]. By administering O₂ at a concentration near to 100% at a pressure of 1.4ATA, arteriolar PpO₂ is approximately 918mmHg; a state of hyperoxia is achieved. This pressure is more than enough to ensure accurate O₂ supply to all tissues, through the diffusion and penetration of O₂ from the plasma to all cells, as indicated by the Krogh model (see Figure 2). In summary, under hyperbaric conditions (at least 1.4ATA), the O₂ penetration (~40µm) required to reach the minimum effective PpO₂ (20mmHg), needed to satisfy cellular functions, is achieved and exceeded considerably. Therefore, the clinical and physiological benefits of HBOT are manifested to 1.4ATA.
Figure 2. Effect of pressure treatment on diffusion profile and the maximum diffusion distance in a homogeneous medium. The $P_{O_2}$ and $O_2$ penetration are estimated according to the distance $R$.

By analogy with drug therapy, HBOT should ensure that $O_2$ concentration is being maintained within the therapeutic window: overpass the minimum $O_2$ threshold needed to fulfill vital functions of aerobic cells, without exceeding high limits of $O_2$ concentrations, avoiding toxic effects due to the excessive production of reactive oxygen species (ROS).

2. **Biochemical events**

At the cellular level and under physiological conditions, $O_2$ is involved in multiple biochemical processes and reactions. The most important of these reactions is the production of energy through oxidative processes that converge in the synthesis of high energy bonds, as adenosine tri-phosphate (ATP). All life processes require energy to be executed.

The main beneficial effects of HBOT are related to $O_2$ transport, hemodynamics and immunological processes [3]. The action mechanism of HBOT is to produce hyperoxia and temporary increase the production of ROS [5]. Thus, it solves adverse conditions
such as hypoxia and edema, and promotes normal physiological responses or responses against infectious and ischemic processes [3]. Additionally to generate ROS and free radicals, HBOT stimulates the expression and activity of antioxidant enzymes, to maintain homeostasis and the redox cellular state (reductive/oxidative) and ensure treatment safety [3, 6].

Among the mechanisms promoted by HBOT in problem wounds, we can include:

- **Vasoconstriction.** This effect is favored by increasing available $\text{O}_2$ in small arteries and capillaries. Vasoconstriction occurs in healthy tissue without deterioration in oxygenation, promoting flow redistribution to hypoperfused areas [3]. Therefore, the vasoconstriction produced is called "non hipoxemic", since it does not counteract the effect of hyperoxia.

**Angiogenesis.** Hyperoxia stimulates neovascularization, or the formation of new vessels, by two different processes: vasculogenesis and angiogenesis [6-8]. Angiogenesis is a regional process, driven by endothelial cells of blood vessels in regions affected by adverse events, injury or local hypoxia. Vasculogenesis is the *de novo* formation of blood vessels, favored by the stimulus of endothelial cells and new blood vessels cells on the formation, migration, recruitment and differentiation of stem or progenitor cells to the site of injury or hypoxia [6].

At a biochemical level, this mechanisms involves several growth factors, transcription factors, hormones and chemical mediators (HIF-1, EPO, VEGF, EGF, PDGF, IL) [5]. For example, sites of neovascularization generate ROS, stimulating the production of transcription factors (HIF-1: hypoxia inducible factor) [6], through HIF-1α and HIF-1β subunits stabilization and dimerization [9]. HIF-1 stimulates the production of growth factors involved in neovascularization, such as VEGF (vascular endothelial growth factor) [6], for migration and differentiation of stem cells to endothelial cells [5], and
erythropoietin (EPO). While hypoxia is the major trigger mechanism of angiogenesis [7], if this condition is prolonged over time, the angiogenesis processes do not persist [7, 10, 11]. In particular, the pro-angiogenic effect triggered by HBOT is mediated by an increase of VEGF production [7], favoring the formation of new vessels after several sessions.

On the other hand, HBOT has effects on bone marrow, modulating the activity of nitric oxide synthase (NOS), which synthesizes nitric oxide (free radical, FR) and is involved in stem cell moving, favoring the healing process [6].

**Cellular immune response against infections.** In adverse conditions such as hypoxia, susceptibility to infections increases. In hyperoxia, some immune cells such as neutrophils or polymorphonuclear cells (PMN) respond to pathogenic noxa exerting its bactericidal action, through ROS and FR production and peroxidase enzymes activity [3]. These chemical mediators damage DNA and oxidize proteins and lipids (lipid peroxidation), inhibiting bacterial metabolism. In this context, the attack against anaerobes is reinforced, because they are unable to produce their toxins in hyperoxic conditions (α-toxins produced during *clostridium perfringens* sporulation in gas gangrene). Furthermore, HBOT exerts synergistic action with some antibiotics facilitating O2-dependent transport through the bacterial cell wall [3].

Notably, HBOT effects on cellular immunity reduces mediated cell injury in ischemic tissues without affecting the immune functions of white blood cells (WBC) (degranulation, phagocytosis), therefore it does not generate immune compromise to the patient [6]. For example, preventive indications of HBOT protects from injury by post-ischemic reperfusion (inhibiting β2-integrins synthesis, responsible for circulating neutrophils adhesion to vessels) [3] and thrombogenic effects (mediated by leukocytes) [5].
**Collagen synthesis.** Collagen is a structural protein synthesized by fibroblasts by complex chemical reactions, including hydroxylation of proline and lysine amino acids. The hydroxylation reaction and cross-linking of the collagen fibers, are favored in the presence of high O\(_2\) concentrations (peptides and propeptides collagen) [5].

**Anti-inflammation and edema reduction.** Vasoconstriction helps to reduce the inflammatory response and therefore to reduce edema [3]. HBOT diminishes production and release of pro-inflammatory cytokynes by neutrophils and monocytes [5].

**Cell proliferation and differentiation.** Collagen synthesis and extracellular matrix formation is produced by fibroblast proliferation, favoring the formation of scar tissue and new vessels, looking to resolve hypoxic conditions and tissue hypoperfusion injury. This effect is also mediated by the increased synthesis of growth factors favored by HBOT through FR and ROS. In addition to stem/progenitor cells and new vessels, components such as collagen are essential in the mainly proliferative and remodeling phases, during the healing process [8].

**Wound healing.** Along with stimuli which promote collagen synthesis and neovascularization, hyperoxia also stimulates the formation of granulation tissue in regions affected by adverse conditions. Through the synergy between these mechanisms, the process of wound healing is accelerated.

**Markers**

The HBOT follow-up includes clinical, biochemical and image studies for each specific pathology, together with general parameters that are affected by HBOT *per se*. These markers are sensitive to different pressures and for different pathologies [12-17]. These biochemical parameters can be classified according to the different hyperoxia mechanisms of action:
- Coagulation and hemostasis: KPTT, protrombin time, RIN, fibrinogen dosage, platelets, hepatic profile [18, 19]
- Acute phase reactant and inflammation markers: PCR, ceruloplasmin, integrin, hematological profile [13, 15]
- Immunity: antibodies, white blood count, neutrophils and lymphocytes
- Oxidative status: reactive O₂ metabolites, MDA, antioxidants (enzymatic: glutation peroxidase, superoxide dismutase, NOS, catalase, mieloperoxidase; non enzymatic: glutation, vitamins (C, A, E)) [12-16]
- Healing and angiogenesis: VEGF, collagen peptides, EPO [7, 13]

3. Trials
Several articles and reviews are available in the literature, including clinical trials, case reports, expert opinions and original research articles describing the effects and benefits of HBOT in patients, laboratory animals and model systems. Among works with patients, most of HBOT results derive from systematic reviews and randomized clinical trials (RCT) for several pathologies and at different pressures. In addition to the applications of this therapy as a first-choice option (acute processes) or as an adjuvant therapy, complementary to other indications, HBOT shows great effectiveness when indicated at early stages and even in a preventive fashion [6, 9]. HBOT is usually indicated by specifying different variables that, together, determine the O₂ dose:
- Treatment pressure
- %O₂ administered (continuous or at intervals)
- Session length: 60-90'
- Total number of sessions
- Daily/weekly frequency of sessions
- Total duration of sessions

In recent years, the treatment at pressures close to the minimum pressure requirement established by the Society of Hyperbaric Medicine (UHMS) [1] has been applied in various pathologies, around 1.4ATA, since it is safer, easier to apply and shows excellent therapeutic efficacy [12].

**Table 1.** Indications and statistics of cases treated with HBOT Revitalair chambers in wounds.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Number of cases</th>
<th>Therapeutic effectiveness</th>
<th>Indicated sessions (average)</th>
<th>Indicated frequency (average)</th>
<th>Sessiones compliance</th>
<th>Patient satisfaction</th>
<th>Sessions length (average)</th>
<th>Patient evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abscess</td>
<td>7</td>
<td>97%</td>
<td>36</td>
<td>3</td>
<td>100%</td>
<td>86%</td>
<td>68 min.</td>
<td>96%</td>
</tr>
<tr>
<td>Vascular disease</td>
<td>10</td>
<td>96%</td>
<td>29</td>
<td>4</td>
<td>90%</td>
<td>100%</td>
<td>66 min.</td>
<td>100%</td>
</tr>
<tr>
<td>Bedsores</td>
<td>2</td>
<td>100%</td>
<td>20</td>
<td>5</td>
<td>100%</td>
<td>100%</td>
<td>60 min.</td>
<td>100%</td>
</tr>
<tr>
<td>Fistula</td>
<td>5</td>
<td>100%</td>
<td>34</td>
<td>4</td>
<td>100%</td>
<td>100%</td>
<td>67 min.</td>
<td>100%</td>
</tr>
<tr>
<td>Wounds</td>
<td>4</td>
<td>97%</td>
<td>30</td>
<td>2</td>
<td>100%</td>
<td>100%</td>
<td>61 min.</td>
<td>94%</td>
</tr>
<tr>
<td>Diabetic foot</td>
<td>3</td>
<td>100%</td>
<td>37</td>
<td>3</td>
<td>100%</td>
<td>100%</td>
<td>67 min.</td>
<td>100%</td>
</tr>
<tr>
<td>Ulcers</td>
<td>104</td>
<td>95%</td>
<td>39</td>
<td>3</td>
<td>95%</td>
<td>96%</td>
<td>67 min.</td>
<td>96%</td>
</tr>
</tbody>
</table>

The following are the prominent applications of HBOT in wounds, including diabetic foot, venous ulcers, burns and post-surgical wounds.

**HBOT in wounds with healing problems**

In particular, HBO treatment is widely used for wounds with healing problems. These include burns, diabetic foot and chronic peripheral vascular obstruction.

Wounds and injuries with vascular involvement generate problems of perfusion, ischemia and hypoxia conditions [20]. Often, the problems in microvasculature (as diabetic micro-angiopathy) persist with conventional treatment, making difficult to heal injuries and wounds [21]. These injuries tend to spread and become infected, and often lead to even more serious complications, leading to amputations.

Among the most commonly treated injuries, diabetic wounds affecting lower limbs respond to HBOT as adjuvant to conventional treatment [22]. The use of HBOT for
diabetic foot is indicated for wounds under an established classification (Wagner grade 3 or higher) [21, 22], with certain characteristics (penetrating ulcer, abscess or gangrene in the tendons, bones or joints, with no measurable signs of healing) [22]. It is postulated that HBO would increase the oxygenation of hypoxic tissues, favoring their healing, as already mentioned in the first part of this report. HBOT acts to promote wound healing through: tissue hyper-oxygenation, vasoconstriction, fibroblast activation, inhibition of production and release of inflammatory cytokines, stimulation of growth factors and neovascularization, antibacterial effect, granulation tissue formation, potentiation of the antibiotic effect and reduction of leukocyte adhesion [23, 24].

In particular, the pro-angiogenic effect is favored by HBO acting on regional growth factors (VEGF), recruitment and differentiation of circulating stem/progenitors cells and on the production of extracellular matrix [7]. In addition, in the case of diabetic patients, HBOT helps in the peripheral utilization of glucose [25]. The treatment efficacy can be assessed from clinical measurement (Doppler, infrared thermography) [26] and transcutaneous O₂ tension (PtcO₂) at the wound edges [27]. In diabetic ulcers, a significant increase in PtcO₂ after HBOT indicates a greater likelihood of viability of the affected limb. It should be noted that HBOT is well tolerated and provides benefits to the patient's quality of life, reducing the number of hospital admissions and morbidity of these patients. In addition, its cost-effectiveness is well documented, considering the reduction of treatment costs, hospital stay, risk of amputation and improvement of productivity of patients with diabetic foot [8, 20, 27-35].

Numerous trials have been carried out to evaluate the effects of HBO in patients with diabetic foot. Results from multiple studies in a significant number of patients showed
high success rate in patients refractory to other treatments, highlighting the role of HBOT for deep chronic infected wounds in patients with diabetes mellitus. In this regard, the efficacy of adjuvant HBOT was assessed through a systematic review [20, 21] for the treatment of chronic ulcers of the lower extremities (diabetic foot ulcers, venous, arterial and pressure ulcers). For diabetic foot, HBOT favors reduction in the risk of major amputation, compared to alternative treatment. In addition, there was a significant improvement in the prospects of healing one year after treatment. For venous ulcer, reduction of wound size was observed. These results should be extended with further trials of high methodological rigor and, in addition, define those patients who can obtain more and greater benefits from HBO.

In acute wounds, such as burns, grafts and implants, it was found that the incorporation of HBOT favors and accelerates healing, reduces the need for additional surgical procedures and tissue necrosis, compared to routine treatment [36]. In these wounds, HBOT also reduces morbidity and mortality, shortens hospital stay and improves the patient’s quality of life [37, 38].

CONCLUSIONS

HBO is successful and widely used as primary or adjuvant therapy in different pathologies. Its effectiveness is based on the generation of hyperoxia, from which multiple physiological benefits are triggered for the patient. Many of the biochemical effects and mechanisms favored by hyperoxia can be evidenced through the monitoring of biochemical markers. These markers are sensitive to the therapeutic action of HBO at different pressures and in different pathologies, showing changes mainly in antioxidant system and anti-inflammatory response.
Given the mechanism of action of HBOT, its application is approved for pathologies of varied origin, framed in different medical specialties. Its use is in constant research and growth phase. There is a great amount and variety of trials describing the effects of HBOT in different specialties and pathologies. Both in daily practice and in the development of clinical trial, it is important to consider, in particular, the duration of each session and the number and frequency of weekly sessions for each specific disease. In wounds with vascular involvement, HBOT has been shown to be useful in resolving ischemia and hypoxia, collaborating in neo-vascularization and healing. Particularly used as adjunctive treatment for proper wound care and, where appropriate, control of the underlying disease, HBOT decreases the morbidity and incidence of infections and amputations, accelerates healing times and reduces wound size.

REFERENCES


