Hyperbaric Oxygen Physiology

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ABSTRACT

The physiologic effects of hyperbaric oxygen (HBO) on various disorders and conditions have been consistently and extensively evaluated through experimental and clinical studies since the 1950s. Each indication of HBO has been the subject to a high level of scrutiny by relevant committees. The current paper provides a brief description of the physiology of HBO through 4 major subheadings: 1- HBO and hypoxia, 2-HBO and infection, 3-HBO and ischemia-reperfusion injury, and 4-HBO and wound healing.

1.0 INTRODUCTION

The physiologic effects of hyperbaric oxygen (HBO) on various disorders and conditions have been consistently and extensively evaluated through experimental and clinical studies since the 1950s. Each indication of HBO has been the subject to a high level of scrutiny by the relevant committees such as the European Underwater and Baromedical Society (EUBS) and Underwater and Hyperbaric Medicine Society (UHMS). As may be observed from table 1, these are dynamic lists that have been subject to changes following cumulative and comprehensive findings from well-designed clinical researches. For instance in 1992 while some indications were removed from the list, several others were added (Table 1). Sudden sensory hearing loss, which in part, is the subject of the current lecture series has been added to the indication list of UHMS in 2011.

Table 1: Accepted and removed indications for HBO according to UHMS

A. Accepted

1. Air or Gas Embolism
2. Carbon Monoxide Poisoning
3. Carbon Monoxide Poisoning Complicated By Cyanide Poisoning
4. Clostridial Myositis and Myonecrosis (Gas Gangrene)
5. Crush Injury, Compartment Syndrome and Other Acute Traumatic Ischemias
6. Decompression Sickness
7. Arterial Insufficiencies:
   - Central Retinal Artery Occlusion (2008)
   - Enhancement of Healing In Selected Problem Wounds
8. Severe Anaemia (1992)
9. Intracranial Abscess (1996)

10. Necrotizing Soft Tissue Infections
11. Osteomyelitis (Refractory)
12. Delayed Radiation Injury (Soft Tissue and Bony Necrosis) (1992)
13. Compromised Grafts and Flaps
14. Acute Thermal Burn Injury (1992)
15. Idiopathic Sudden Sensorineural Hearing Loss (2011)

B. Removed

1. Refractory mycosis (1983)
2. Cyanide poisoning (1992)
3. Acute cerebral edema (1992)

The physiology behind each of these indications will be classified and discussed under 4 main topics.

i. *HBO and hypoxia: Hypoxia, at least in part, accounts for the pathophysiology of each of the conditions listed in the indication lists.*

ii. *HBO and infection*

   a. *Gas Gangrene*

   b. *Necrotizing Soft Tissue Infections*

   c. *Intracranial Abscess*

   d. *Osteomyelitis (Refractory)*

iii. *HBO and ischemia-reperfusion injury*

   a. *Acute Traumatic Ischemias*

   b. *Compromised Grafts and Flaps*

   c. *Carbon Monoxide Poisoning*

iv. *HBO and wound healing*

   a. *Enhancement of Healing In Selected Problem Wounds*

   b. *Acute Thermal Burn Injury*

2.0 HBO AND HYPOXIA

Physiologic effects of HBO on hypoxia may be briefly described by the Henry's Law which states that:

"At a constant temperature, the amount of a given gas (O_2) that dissolves in a given type and volume of liquid (plasma) is directly proportional to the partial pressure of that gas in equilibrium with that liquid."

Increasing the ambient pressure, as with HBO, increases the amount of oxygen dissolved in plasma. The partial pressure of oxygen (pO_2) under various oxygen concentrations are shown in table 1.

Table 1: The partial pressure of oxygen under various oxygen concentrations.

Inhaled pO_2 (atm)	*Arterial pO_2 (mmHg)*	*Tissue pO_2 (mmHg)*
0.21	**90 ± 9**	**41 ± 10**
1.0	**625 ± 23**	**76 ± 45**
2.0	**1356 ± 28**	**280 ± 50**
2.5	**1700**	**348**

HBO not only increases the arterial pO2, but also the total arterial O2 concentration (CaO2). Arterial O2 content is the sum of hemoglobin (Hb)-bound O2 and dissolved O2. This relation is formularized as follows:

$$\mathbf{Ca\ O_2 = (Hb \times 1.34 \times SaO_2) + (PaO_2 \times 0.003)}$$

CaO_2 = Arterial O_2 concentration (mL O_2/ 100mL)	
Hb = (g/100mL)	**PaO_2** = Arterial blood pO2 (mmHg)
1.34 = O_2 concentration per 1g of Hb (mL)	**0.003** = Solubility constant for O_2
SaO_2 = Hb saturation (%)	

When breathing air, i.e. about 20% oxygen at sea level (1 atm abs) assuming a 15 g/100mL Hb concentration, total arterial O_2 concentration is around 20,4 ml /dl (20,1 ml/dl Hb-bound oxygen and 0,32 ml /dl dissolved oxygen) (Figure 1). As may be observed in figure 2, while the Hb-bound oxygen concentration remains constant, the concentration of dissolved oxygen displays a gradual increase as the percentage of oxygen in ambient air and/or the pressure of the ambient air is increased.

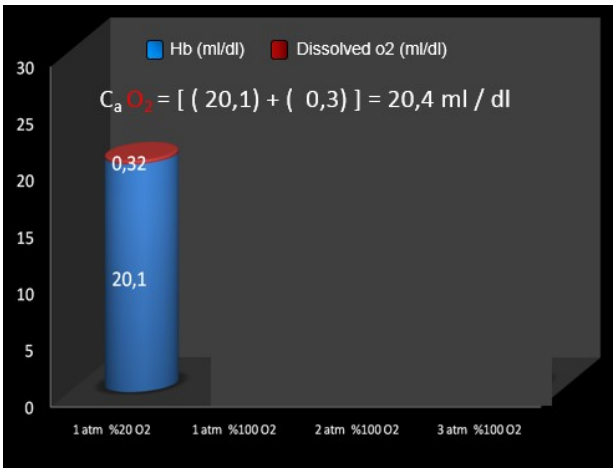

Figure 1: Arterial oxygen concentration at 1 atm abs pressure when breathing air and assuming a 15g/mL Hb concentration. Hb-bound oxygen (20,1 ml/dl, blue color) and dissolved oxygen (0,32 ml / dl, red color) together, sum up to 20,4 ml/dl.

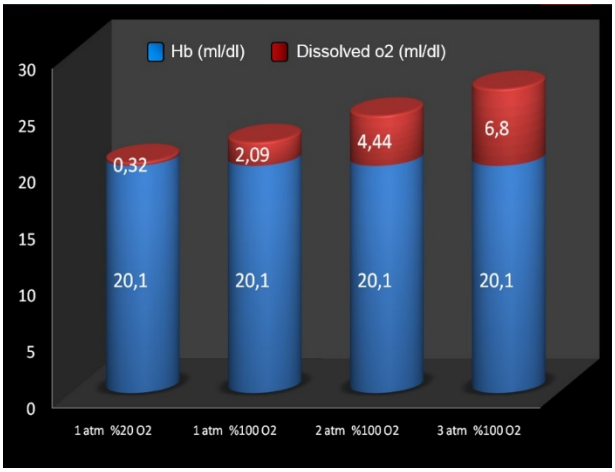

Figure 2: As the percentage of oxygen in ambient air and/or the pressure of the ambient air is increased Hb-bound oxygen concentration (blue color) remains constant, but the concentration of dissolved oxygen displays a gradual increase (red color).

While CaO_2 refers to arterial O_2 concentration in 100mL, the total amount of oxygen circulated to cells is described as "**oxygen delivery (DO_2)**". **DO_2** is formularized as follows:

$$DO_2 = CO \times CaO_2$$

DO_2	= O_2 delivery (mL/min)
CO	= Cardiac output (mL/min)
CaO_2	= Arterial O_2 concentration (mL O_2/ 100mL)

Assuming a 5L/min CO, **DO_2** would be 1.02 L/min, i.e. almost 20% of the total output.

The amount of O_2 consumption in a given time by any tissue, usually represented as VO_2, is the difference of O_2 concentration between the arterial and venous side of that tissue (Arteriovenous O_2 difference) multiplied by the cardiac output. VO_2 may be formularized as follows:

$$VO_2 = CO \times (Ca\,[O_2] - Cv\,[O_2])$$

VO_2	= O_2 consumption at a given time (mL/min)
CO	= Cardiac output (mL/min)
CaO_2	= Arterial O_2 concentration (mL O_2/ 100mL)
CvO_2	= Venous O_2 concentration (mL O_2/ 100mL)

To conclude dissolved oxygen concentration when breathing 100% O_2 at 3 atm abs, assuming a 15g/mL Hb concentration is around 6,8ml /dl. This amount of oxygen has been shown to be sufficient for the basic metabolic needs ($AVDO_2$ or VO_2) of an individual at rest with normal cardiac output (1) (Figure 3).

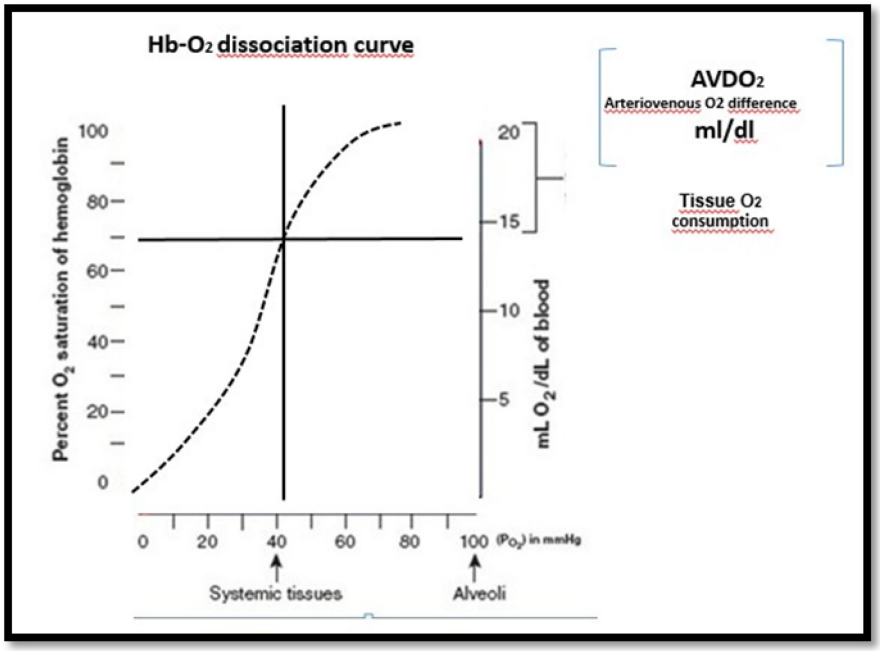

Figure 3: The oxyhemoglobin dissociation curve. At the arterial side, when the Hb is fully saturated and when pO_2 is above 90mmHg total arterial O_2 concentration is around 20, 4 ml/dl. Note that at the venous side, saturation is 75%, pO_2 around 40mmHg and venous oxygen concentration approximately 14 ml/dl. The difference in CaO_2 between the arterial and venous sides of any given tissue is equal to O_2 consumption of that tissue. This consumption, which is around 6ml/dl for the majority of tissues, is below the dissolved O_2 concentration achieved by HBO at 3 atm.

3.0 HBO AND OXIDATIVE STRESS

Reactive oxygen (ROS) and nitrogen (RNS) species, provided that they are maintained in the physiologic range, play a major role in multiple biologic pathways. They function as signal transduction molecules via transcription factors. ROS, RNS and antioxidants function normally in harmony. Modifying this equilibrium in favor of ROS, however, may have dramatic consequences, usually described as oxidative damage.

One of the major expressions of concern regarding HBO therapy is ROS production. HBO, indeed, promotes ROS production, yet this production, under therapeutic HBO doses, remain within the antioxidant defense mechanism capacity. Moreover, HBO potentiates the function of antioxidant enzymes and thereby protects cells from oxidative damage (2). Oxidative damage occurs when the production of ROS and/or RNS overcomes the protective capacity of the antioxidant systems.

Nitric oxide (NO), which is among these free radicals has gained popularity in recent decades and has been the subject of many studies.

NO, at low levels:

i. *Causes smooth muscle relaxation.*

ii. *Reduces the expression of "P selectin", an adhesion molecule implicated in neutrophil-platelet adhesion.*

iii. *Inhibits the production of "Intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1)", adhesion molecules involved in neutrophil- endothelium adhesion.*

Adhesion molecules such as, ICAM, VCAM and E-selectin are not continuously expressed but rather generated in response to injury, an inflammatory stimulus such as cytokine, an endotoxin or ROS themselves. Nuclear factor kappa-light-chain (NFkB) which is involved in inflammatory processes is a strong stimulus for their synthesis.

It has been consistently shown that NO plays a major role in HBO related mechanisms. NO can interact with NFkB and prevent its translocation into the nucleus and prevent the transcription of adhesion molecules. HBO, through NO synthesis, reduces translocation and activation of NFkB.

In ischemic conditions, NO synthesis from arginine is interrupted and NO dependent protective effects are impaired. At large amounts, NO interacts with superoxide (O_2^-), forms the peroxynitrit ($ONOO^-$) molecule and paradoxically causes radical damage. Nitration through $ONOO^-$ basically causes:

i. *Lipid peroxidation*

ii. *Protein denaturation*

iii. *DNA damage*

Ischemia reperfusion injury (IRI) is strongly associated with increased *oxidative stress* and is implicated in the pathogenesis of several disorders such as crush syndrome, myocardial injury, and carbon monoxide poisoning. In IRI, tissue damage is, paradoxically, greater than damage caused by ischemia alone. Ischemia causes endothelial metabolic dysfunction, and impairs mitochondrial function. Therefore during reperfusion O_2 no longer serves as a metabolic substrate but, instead, functions as the source of increased superoxide production. Superoxide and related radicals stimulate an injury response which recruits polymorphonuclear leukocytes (PMNL) towards the injured endothelium from where they migrate into the damaged tissue. Resident cells, such as the endothelial cells, are responsible for the early rise in ROS. ROS formation, in turn, leads to the recruitment of further neutrophils which cause a more significant and sustained rise in ROS production (Figure 4, 5, 6).

Xanthine dehydrogenase (XDH) and xanthine oxidase (XO) are both involved in the oxidative metabolism of purines. PMNL-derived proteases react with XDH to form XO which is the major source of ROS, particularly superoxide, production in inflammatory conditions (Figure 7).

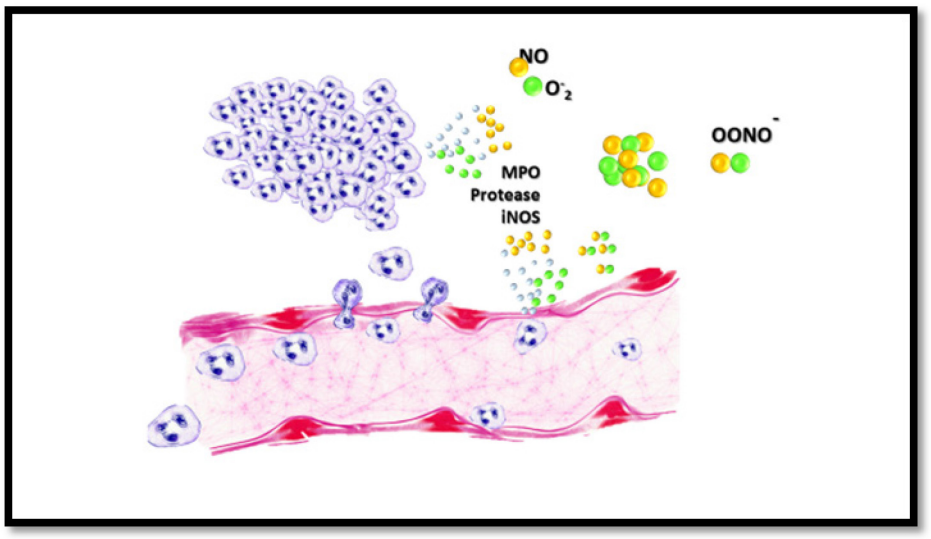

Figure 5: Extravasation (diapedesis) and aggregation of PMNL at the injury site following rolling and adhesion to the endothelium. PMNL-endothelial cell adhesion is a strong stimulus for the release of multiple deleterious enzymes and ROS which, together, cause further tissue damage. Aggregation and activation of PMNL leads to the overproduction of NO and O_2^- which form the $ONOO^-$.

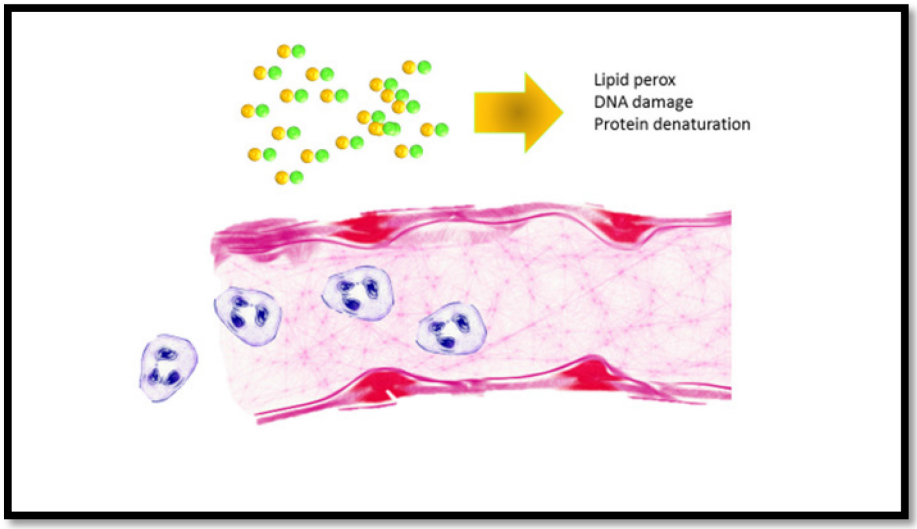

Figure 6: $ONOO^-$ is a strong nitrating agent which basically causes lipid peroxidation, protein denaturation and DNA damage. PMNL-endothelial cell interactions are believed to be, at least in part, responsible for pathologies related to IRI such as brain IRI, myocardial injury and CO mediated injury.

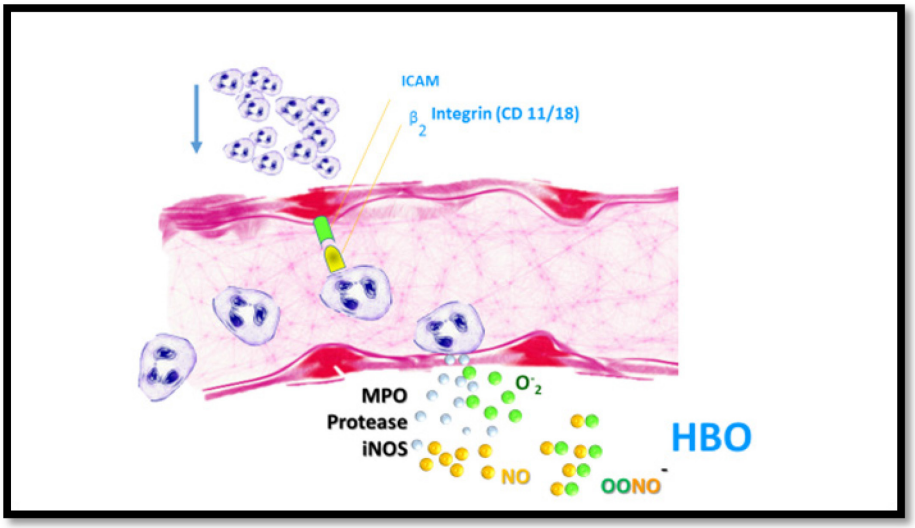

Figure 7: PMNL-endothelial cell adhesion and PMNL aggregation is reduced with HBO. This is achieved through actin S-nitrosylation which inhibits neutrophil-β_2 integrin function (3). Additionally, HBO reduces ICAM-1 and VCAM-1 expression from endothelial cells in IRI.

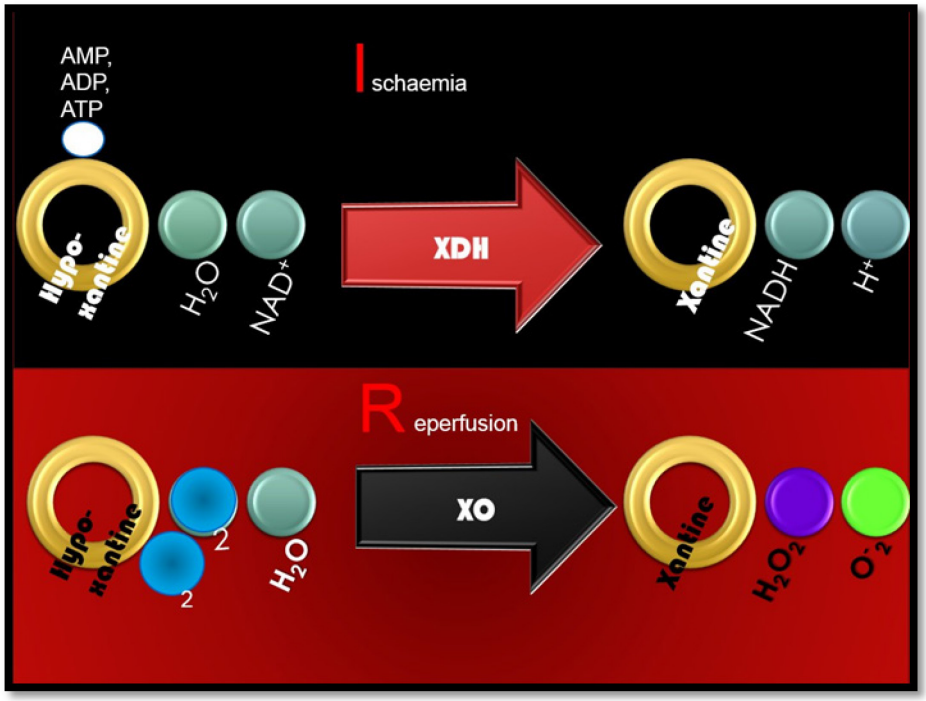

Figure 7: XDH and XO catalyze the oxidation of hypoxanthine to xanthine. They play an important role in purine catabolism. XDH can be converted to XO either by reversible sulfhydryl oxidation (Nytration) or by irreversible proteolytic modification (PMNL derived proteases). Note the production of ROS (hydrogen peroxide and superoxide) in the reperfusion phase of IRI.

4.0 HBO AND INFECTION

Innate oxidative immunity involves the killing of microorganisms through O_2 and ROS; particularly the highly reactive hydroxyl radical (·OH) (4). PMNL and macrophages require NADPH oxidase; a critical enzyme that produces ROS, to kill phagocytized bacteria, through a process termed the oxidative burst. During oxidative burst oxygen consumption is increased by 25-50 fold and therefore this process requires additional oxygen resources. Oxidative burst lasts until all bacteria are killed (30-60 min). The significance of oxidative burst in bacterial killing has been demonstrated during the course of elucidating the pathogenesis of chronic granulomatous disease (CGD). Patients with CGD lack the NADPH oxidase enzyme and therefore are subject to recurrent infections. In brief, oxidative burst is an O_2 and ROS dependent critical step in bacterial killing through phagocytosis. HBO treatment increases ROS production, provides additional resource for oxidative burst and thereby augments PMNL bacterial killing (Figure 8).

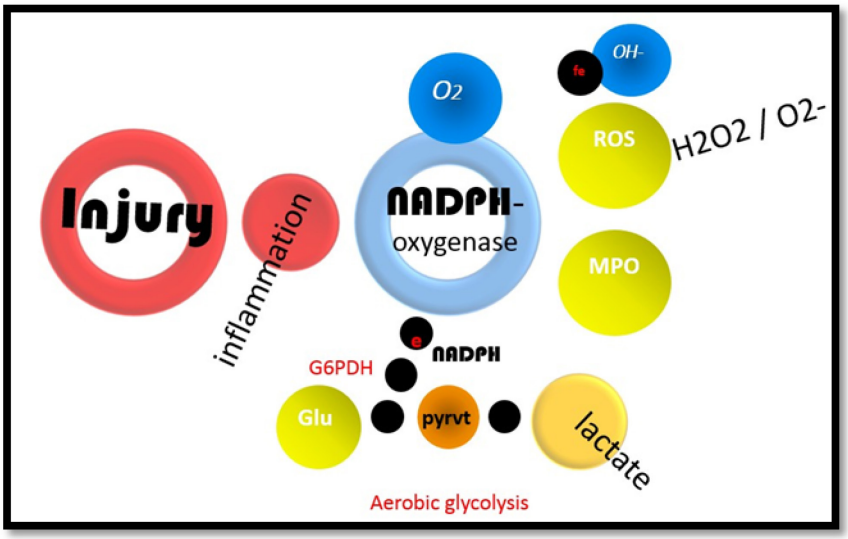

Figure 8: The role of NADPH-oxygenase in oxidative burst.

Among many infectious disorders, HBO is particularly used in the treatment of necrotizing soft tissue infections (Figure 9).

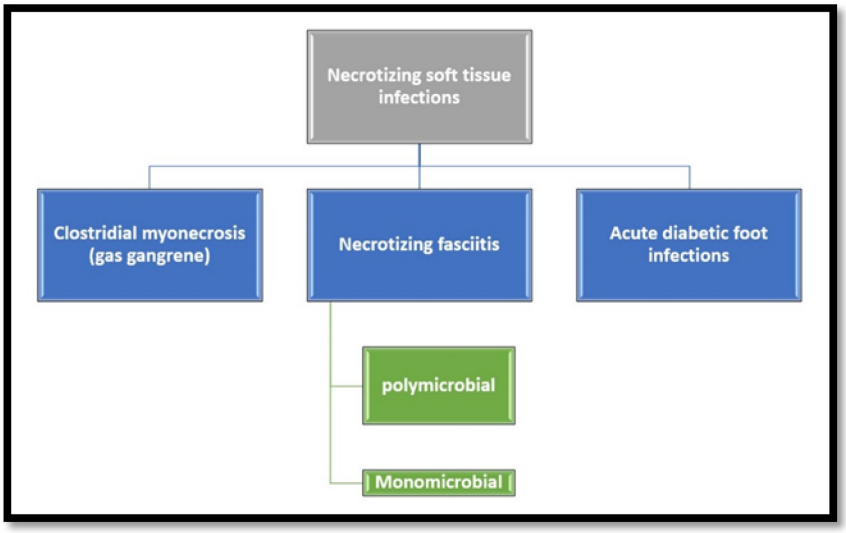

Figure 9: Classification of necrotizing soft tissue infections

Necrotic tissue is, particularly, an ideal focus for the growth of anaerobic organism. Devitalized tissue further promotes the growth of anaerobes and a vicious cycle ensues.

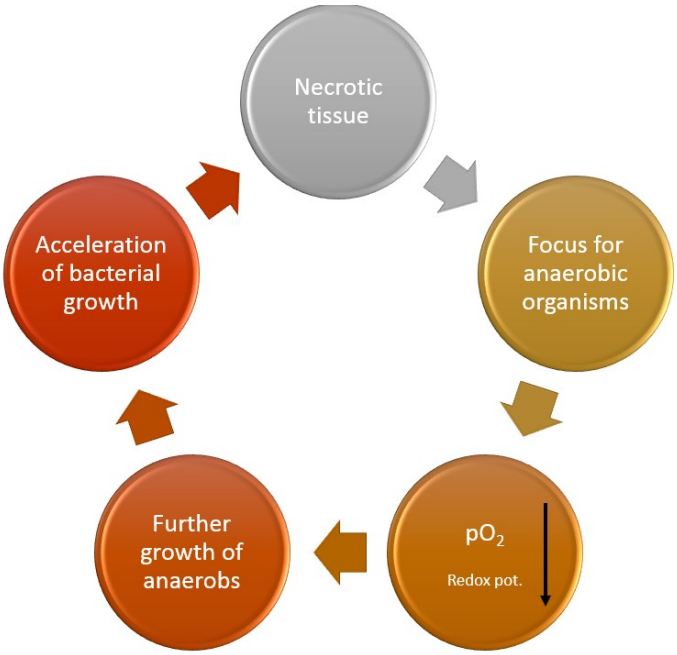

Figure 10: The vicious cycle implicated in the pathogenesis of necrotizing soft tissue infections.

Effects of HBO in the management of clostridial myonecrosis, i.e. gas gangrene:

i. *Causes direct toxicity on anaerobes*

ii. *Slows down clostridial proliferation*

iii. *Stops α-toxin production*

iv. *Enhances oxidative killing*

v. *Fastens demarcation*

vi. *Decreases the amount of tissue loss*

vii. *Improves RED-OX potential*

viii. *Produces synergistic effect on antibiotic efficiency through changing redox state.*

The half-life of Alfa-toxin is short and therefore requires continuous production to cause damage. Using HBO stops α-toxin production. Additional benefits of HBO in necrotizing soft tissue infections:

i. *Reduces surgical site infections*

ii. *Resolves edema via vasoconstriction*

iii. *Improves wound healing through angiogenesis and fibroblast proliferation*

5.0 HBO AND WOUND HEALING

Injury disrupts vascular and lymphatic vessels and leads to edema and hypoxia (5). While acute hypoxia triggers healing pathways particularly through ROS, chronic hypoxia, impairs wound healing and increases the risk of infection.

Chronic hypoxia and ischemia causes:

i. *Delayed wound healing*

ii. *Impaired granulation and epithelialization*

iii. *Impaired leukocyte functioning*

iv. *Impaired antibiotic efficacy*

v. *Poor antibiotic delivery*

Increased oxygen partial pressure in the wound milieu, on the other hand, provides:

i. *Tissue hyperoxygenation*

   a. *Increased diffusion distance*

   b. *Improved mitochondrial function / aerobic metabolism*

ii. *Vasoconstriction and anti-edema*

iii. *Neovascularization*

   a. *Angiogenesis*

   b. *Vasculogenesis*

Chronic hypoxia speeds down collagen synthesis from fibroblasts. While fibroblast proliferation requires a minimum of 15 mmHg pO2, maximum fibroblast proliferation is attained at a pO2 of 1875 mmHg, a level that may be attained with HBO at 2.4 atm abs pressure. Collagen synthesis, likewise, requires O2. Prolin and lysin hydroxylation is a key step in collagen synthesis which is induced by the O_2 dependent enzyme prolyl hydroxylase.

The term neovascularization describes new vessel formation either through budding from residence endothelial cells (angiogenesis) or de novo formation from stem/progenitor cells (SPC) (vasculogenesis). Hypoxia inducible factor -1α (HIF-1α), which is expressed during hypoxia, stimulates transcription of genes that are involved in neovascularization such as vascular endothelial growth factor (VEGF) and stromal derived factor (SDF). HBO augments stem cell function by oxidative stress phenomenon that involves HIFs. HBO elevates HIF-1α levels via an increase in the antioxidant thioredoxin (6) .

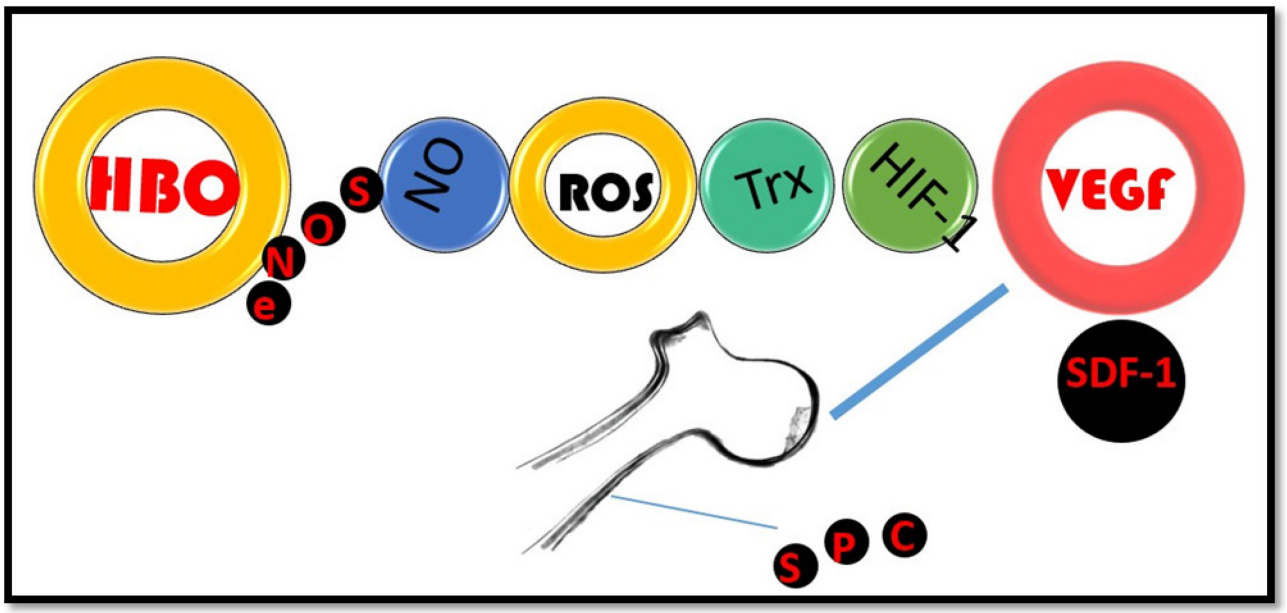

Figure 11: Angiogenesis: HBO induced oxidative stress promotes the production of the antioxidant thioredoxin (7). Thioredoxin, thereafter, induces the expression and activation of HIFs (8). Finally, HIFs stimulate neovascularization through increased synthesis of VEGF and SDF-1 levels.

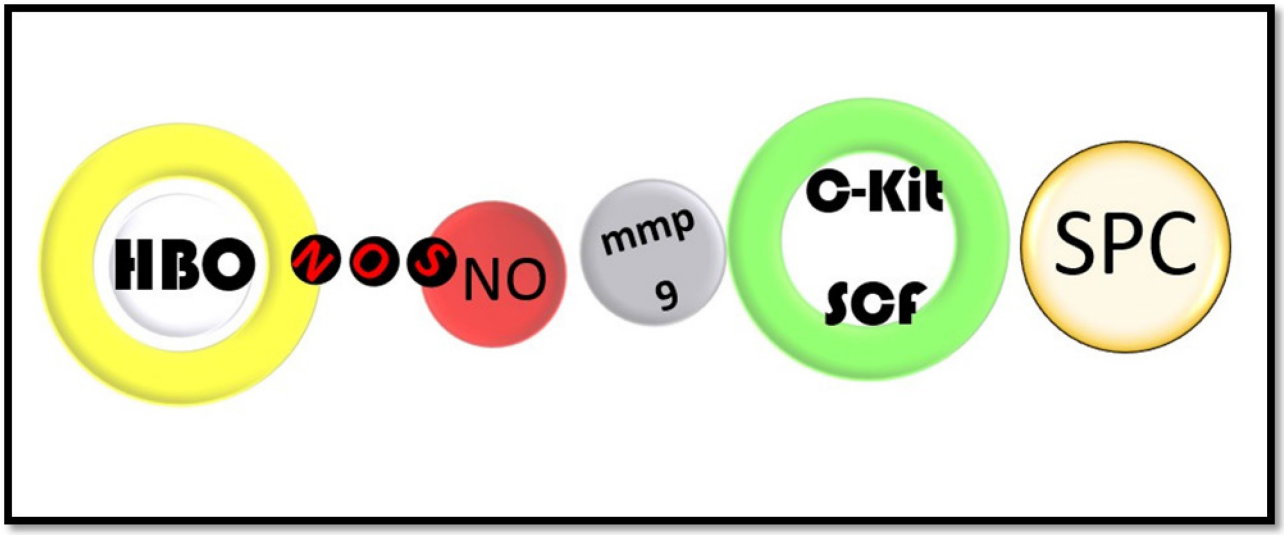

Figure 12: Vasculogenesis: De novo vessel formation from SPC which are mobilized from the bone marrow to the wound milieu. HBO_2 plays a significant role in vasculogenesis by triggering bone marrow SPCs mobilization via NO synthesis (9). NO, stimulates SPC mobilization through the cKit ligand (stem cell factor, SCF) (10).

6.0. REFERENCES

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