The Effects of Hyperbaric Oxygen Therapy to Regulate Nitric Oxide Production on Wound Healing

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Abstract

Hyperbaric oxygen therapy (HBOT) involves inhaling 100% oxygen at greater than one atmosphere absolute (ATA) in a pressurized chamber. HBOT increases the amount of oxygen that is carried in the plasma. As adjunctive therapy HBOT has been demonstrated to increase wound angiogenesis and granulation tissue formation and accelerate wound closure. One of the properties of HBOT is an anti-inflammatory effect and correlated with increased wound Nitric Oxide Production can enhanced wound healing. The aim of this paper to describe effects HBOT mediates increased nitric oxide production on wound healing. Effect HBOT on wound healing increased nitric oxide.

Key worlds: hyperbaric oxygen, nitric oxide, anti-inflammatory, wound healing

INTRODUCTION

Hyperbaric Oxygen Therapy (HBOT) is one of in medical treatments that uses high pressure and oxygen concentration. Patients breathe 100% pure oxygen at a air pressure of more than 1 Absolute atmosphere (ATA) in the high-pressure air chamber. HBOT can increase the amount of dissolved oxygen in such a way in order to reach a state where practically oxygen demand can be fulfilled without the use of oxygen that bound to hemoglobin (Thom, 2011). Dissolved oxygen is very important compared to the form of oxygen that is bound to hemoglobin, more easily consumed by tissues through the direct diffusion. (Mathieu, 2006, Thom, 2011)

Nitric Oxide (NO) is a messenger molecule that may play a role in various physiological and pathological functions in the body (Pacher, 2007). NO compounds showed that multiple modulating effects on inflammation, plays an important role in the regulation of the immune response affects every stage of the development of inflammation. NO dose-dependent effects, in low levels of NO are synthesized constitutively, works on nerve transmission in the regulation of the movement of the
digestive system, regional blood flow, neuroendocrine function (Billack, 2006, Pacher, 2007). At high levels NO acts as a regulator and also serve as effectors during inflammation and infection. NO has a regulatory effect on the immune system, including interactions in signaling systems. This resulted in the modification of the activity of transcription factors and modulate the expression of many other mediators (Guzik, 2003).

Hyperbaric Oxygen Therapy

HBO therapy has been widely used for wound healing in diabetic foot gangrene, burns, ischemia and as an adjuvant therapy in various diseases. In process of wound healing and inflammation it has showed lowered number of inflammatory cytokines IL-1β and TNF-α, reduction of edema (Page, 2007), increase number of the anti-inflammatory cytokine IL-10 (Buras, 2006), the formation of VEGF, TGF-β, PGE-lowering 2 (Al-Wail, 2006), increased level of antioxidant superoxide dismutase, heme oxygenase (HO-1) (Speit, 2000), suppressed NADPH and apoptosis (Zhang, 2008).

The effect of hyperbaric oxygen on the body relies on two modalities: 1). physical factors associated with environmental changes from a normal environment (normobaric) (1 ATM) to a hyperbaric environment (more than 1 ATM) is a mechanical effect of modality, 2). increasing the partial pressure of oxygenation in the blood or tissues that provides many therapeutic effects of hyperbaric oxygen (Bitterman, 2007, Thom, 2011:).

Oxygen transports from the lungs to the tissues in two ways, through attachment with hemoglobin and dissolved in plasma (Wright, 2003; Mathieu, 2006). Oxygen that bounds to hemoglobin is the main oxygen transport by red blood cells in the form of oxyhemoglobin (HbO2). When the alveolar partial pressure reached 200 mm Hg, then became a full saturation of hemoglobin with oxygen. If a person breathes in the sea level (750 mm Hg), only 1.5 percent of the oxygen carried by the blood in the form of dissolved in the plasma. Transport of oxygen in the plasma is the key of hyperbaric oxygen effect on tissues blood perfusion in tissue, although less well (Mathieu, 2006, Thom, 2011).

The Increasing of φ  in the inspiration partial pressure, can increase the amount of dissolved oxygen in the plasma. When the air pressure inside the chamber improved, then the partial pressure of oxygen in alveolar be increased, proportional to the magnitude of increased environmental stress or pressure in the chamber. Furthermore
oxygen pressure in alveolar will an increase in the partial pressure of dissolved in oxygen the plasma. It is the principle of treatment OHB (Hart, 2005; Mathieu, 2006).

Pharmacological effects of HBOT Oxygen are shown to be effective in the event of a state of pathological changes and improvements in clinical status of patients. Oxygen Hyperbaric is used as a drug, has a therapeutic index of minimum effective concentration and the concentration of minimum toxicity. (Prince, 2004 Marthieu, 2006). At OHB pressure therapy used was 2-3 ATA, since it was reported that the administration is less than 3 ATA is an effective dose and has minimal toxicity effects (Wright, 2001; Smith, 2005)

**Nitric Oxide**

A free radical gas, NO is synthesized from the amino acid L-arginine by the enzyme nitric oxide synthase (NOS) synthesizes NO by oxidizing five electrons from the guanidino nitrogen of L-arginine (L-Arg). Oxidation of L-arginine to L-citrulline happen through monooxygenation reaction produces NO-hydroxy-L-arginine (NOHLA) as intermediates. 2 mol O2 and 1.5 mol NADPH is used for the formation of the NO molecule.

\[
\text{L-Arginin} + \text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow \text{NOHLA} + \text{NADP}^+ + \text{H}_2\text{O}
\]

\[
\text{NOHLA} + \frac{1}{2} \text{NADPH} + \frac{1}{2} \text{H}^+ + \text{O}_2 \rightarrow \text{L-citrulline} + \frac{1}{2} \text{NADP}^+ + \text{NO} + \text{H}_2\text{O}
\]

NO physiological functions such as oxidant signaling to inflammation and infection processes and improvement (Guzik, 2003) and functions as a cellular signaling (cGMP). Other physiological functions act as mediator in healing process, vasodilation of blood vessels by inducing relaxation of smooth muscle, inhibits leukocyte adhesion and activation, inhibits platelet aggregation (thrombus); stimulates glucose infusion in muscle cells, maintaining microvascular circulation, a critical factor in wound healing and stem cells (Billack 2006).

NO has dose-dependent effects, low levels of NO work on nerve transmission in the regulation of the movement of the digestive system, local blood flow, neuro endocrine function (Billack, 2006). At high levels of NO that acts as a regulator, as effectors during inflammation and infection. Effector function of which is the effect
of toxicity to microorganisms, were found on the pathogenesis of tissue damage caused by inflammation. NO has a regulatory effect on the immune system, including interactions in signaling systems. This resulted in the modification of the activity of transcription factors and modulate the expression of many other mediators (Guzik, 2003).

Physiologically, NO can react with one element or biological compounds in some ways: 1) NO diffuses into vascular cells and bind to the enzyme guanylate cyclase (GC), this bond will trigger the process of vasodilatation; 2) NO can react with oxygen to form stable nitrates and nitrates which can be measured in serum, urine, or saliva. 3) NO can bind to sulfur (S) which is contained in certain amino acids such as cysteine, and other biological compounds such as glutathione which are known as antioxidants (Packer, 2009).

The binding of the molecule in produces nitrosothiol formation. NO which is released from nitrosothiol resulting biological responses such as smooth muscle relaxation and vasodilation (Burke, 2003).
Hemoglobin is a protein in red blood cells and is composed of two alpha chains and two beta chains. Hemoglobin is able to carry oxygen to the tissues, consists of a beta chain of the amino acid cysteine (containing S) which can bind to NO will be released together with oxygen (Burke, 2003). Increased levels of NO can be happened due to bacterial invasion, this response was associated with an increase in intracellular calcium and activation of protein kinase C. NO as a mediator of tissue repair processes can lead to a variety of healing wounds such as increased collagen deposition, cell migration, vasodilatation, platelet aggregation; inhibits leukocyte adhesion in endothelial cells; modulates endothelial proliferation and apoptosis, triggering immunodulation and antitoksicity bacteria (Billack, 2006). This is due to the function of NO signaling in the cytotoxicity cells that stimulate wound healing by increasing oxygen levels in the tissue and inflammatory mediators for tissue repair and matrix growth and remodeling (Guzik, 2003).

Discussion

Oxygen is a cofactor for the enzyme activity of nitric oxide synthase / NOS and the presence of oxygen is necessary for the production of NO. Reduced or insufficient oxygen will decrease the production of NO because NOS enzymes are not functioning normally (Pandolfi, 2007).

One of the main channels which is important is through the regulation of NF-κB activation. On cells that are undergoing stimulation, IκB kinase enzyme-complex that resulted ubiquitination fosforilasition IκB and degradation by the proteosome. Then dissociation between the inhibitor κB (IκB) with NF-κB and resulted in NF-κB to the nucleus to activate transcription of the iNOS gene (Baker, 2011). Increased iNOS in response to macrophages at hyperbaric oxygen via interferon γ / IFN-γ is secreted by Th 1 cells (Lahti A. 2004). Cytokines IFN-γ induces macrophage cells to secrete anti-inflammatory cytokines. Another theory suggested that increased ROS would increase NF-κB which in turn will express iNOS gene. INOS enzyme produced will catalyze L-arginine with the help of oxygen, NADPH, produce endogenous NO, NADP and sirutilin (Lahti A. 2004)

Stimulation of oxygen will induce heat shock protein HSP32 (heme oksigenase/HO-1) and HSP 70 in mitochondria that protects cells against death (Mendoza M, 2013). Anti-apoptotic mechanism effect has two possibilities. First direct suppression of the activation of the signal transduction protein caspase family, both involving precursor protein chaperones that led mitochondria by HSP. This
action controls membrane permeability and function because of prevents the release of cytochrome c that is required for activation.

Another possibility is that the induction of HSP-32 will catalyze endogenous heme to CO, biliverdin and Fe2+ (Calabrese, 2006). The role of endogenous CO is known as antiinflammatory, cytoprotective especially against hyperoxia exposure. Fe2+ inhibits the synthesis of iNOS at the transcriptional level, and biliverdin forms bilirubin which is a potent antioxidant (Calabrese, 2006). Recent research has shown that bilirubin and biliverdin have strong antioxidant activity against hydrogen peroxide, peroxyl radicals, hydroxyl radicals. Bilirubin and biliverdin to release NO, nitrosil and prevent an increase in thiol. OH-1 effects due to NO and thiol role in the cellular defense mechanisms against NO, so suspected that the bile pigment has a potential function of the uncontrolled production of NO (Calabrese, 2006).

Hyperbaric oxygen therapy increases ROS with the body's response to form HSP-70 as a protection against the onset of oxidative stress. HSP-70 acts to prevent maldeveloping known, aggregation and anti-apoptotic agents (Mendoza M, 2013). The results have demonstrated the effect of hyperbaric oxygen showed an increase in the Superoxide Dismutase enzyme levels in patients with controlled diabetes were significantly (Rossignol, 2012). From the results of some research on the above, it was concluded that hyperbaric oxygen therapeutic dose between 1-3 ATA even result in the formation of ROS or RNS, but does not result in adverse effects due to accompanied by an increase in endogenous antioxidant.

Increased iNOS is not necessarily an adverse effect because NO can has two functions of the cell. In small amounts acts as an antibacterial and antiapoptosis but in large amounts can cause toxicity and apoptosis in cells. Hyperbaric oxygen therapy triggers the expression of Bcl-2 and increase the bioavailability of intracellular oxygen is used to improve the integrity of mitochondria and decrease in mitochondrial apoptosis pathway activation (Zhang QI, 2008).

Hyperbaric Oxygen Therapy increases iNOS catalyzing the synthesis of arginine to form NO. Then NO increases the expression of heme oxygenase (HO-1) (Murder, 2005; Mendoza, 2013). Induction of HO-1 increases antioxidants act as anti-inflammatory, anti-apoptotic, and anti-proliferation (Converso, 2006). OHB catalyst is thought to contribute to the degradation of heme oxidation to biliverdin with the release of carbon monoxide (CO) and iron. Anti proliferative function of HO-1, an antioxidant known to form CO pro oxidant and function convert cell
cytotoxic and anti-inflammatory effects that lower levels of IL-1β, TNF-α, IL-6 and extend the LPS-induced cell survival (Rossignol DA. 2012).

![Diagram](image)

**Figure**

Schematic picture of interaction between pathways regulation of iNOS and HO-1

Activation of iNOS produces NO from L-arginine. iNOS derived nitric oxide positively (1) modulates heme metabolizing enzyme HO-1. Activation of HO-1 resulted in the catabolism of heme to produce biliverdin, carbon monoxide (CO), and iron. Iron lowers iNOS transcription, CO inhibited iNOS activity. Biliverdin and bilirubin are antioxidants (Murder, 2005, Ryter, 2007).

NOS expression due to hyperbaric oxygen therapy, there are several possibilities. Some factors that may increase the bioactivity of NO hyperoxia, in addition to arginine supplementation, topical NO secretion, increased/decreased antioxidant activity, and oxidative stress. Padgonkar et al using cultured epithelial cell models, suggesting that the OHB treatment increased the expression of heme oxygenase (HO-1) (Murder, 2005). HO-1 levels increased by induction of oxidative stress, HO-2 are expressed constitutively. Induction of HO-1 as a boost antioxidant defenses in mice that have been proven role as an anti-inflammatory, anti-apoptotic, and anti-proliferation, and is now known to have a positive effect on various diseases such as atherosclerosis and sepsis (Converso, 2006). Increased activity of HO-1 reported lower epithelial cell apoptosis and decrease superoxide anion formation in diabetic rats. Increase in HO-1 in diabetic rats was associated with decreased iNOS (Zhang, 2008).
Iron and biliverdin and bilirubin have potential for intracellular redox homeostasis. Biliverdin and bilirubin are known to have an effect on serum antioxidants, iron are usually involved in the synthesis of pro-oxidant (Ryter, 2007). CO also has been demonstrated in cultured cells and endothelial cells is protective role against apoptosis, by inhibiting TNF-α in fibroblasts and endothelial cells of mice. CO effects specific to anti-apoptosis associated with reduced caspase activation and antiapoptosis resistance, Bcl members. Antiapoptosis in this condition is associated with decreased activation of extracellular signal (Ryter, 2007).

Hyperbaric oxygen therapy increased the expression of HO-1 in human lymphocytes. Lymphocytes induce HO-1 and then the adaptive protection so that exposure to hyperbaric oxygen did not induce DNA damage, and does not lead to the development of adaptive protection. Previous studies showed that LPS induces iNOS (Rothfuss, 2002). Kitamura et al proved that nitric oxide (NO) can trigger the expression of HO-1. Another study Lin et al demonstrated an association between HO-1 with resistance to the induction of iNOS expression in macrophages LPS. The data indicate there is a relationship between iNOS with HO-1 Decrease in antioxidant effect on ROS, cell signaling molecules that affect the regulation of iNOS gene transcription. (Converso, 2006, Lin, 2008).

**Conclusion**

Hyperbaric therapy as a therapeutic wound healing, therapy through the regulation of NO that is catalyzed by iNOS the enzyme function as anti-oxidants, inflammation, anti-proliferation and anti-apoptosis which would induce wound repair. Activation of iNOS produces NO from L-arginine. HO-1 modulates iNOS. Activation of HO-1 resulted in the catabolism of heme to produce biliverdin, carbon monoxide (CO), and iron. Iron lowers iNOS transcription, and CO inhibited iNOS activity. Biliverdin and bilirubin are antioxidants.
Reference
