

PROPOSED MECHANISMS OF ACTION OF SELECTED ANTIOXIDANT DEFENCES INDUCED BY HYPERBARIC OXYGEN THERAPY

Minireview

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Abstract

Hyperbaric oxygen therapy (HBOT) has been proved as an important tool in healing of CO poisoning, necrosis, ischemic wounds, inflammatory diseases or brain injuries. Reactive oxygen species (ROS) play a key role in healing potential of HBOT however potential side effects must be taken into account. Equally important is also duration of HBOT. We can conclude, that despite several decades of HBOT use in clinical therapy, there are still not known it's detailed mechanisms of action on the molecular level and considerable part of information comes of animal experiments. Apart from the results of basic research is necessary to obtain a larger volume of complex data from clinical trials of HBOT.

Keywords: Hyperbaric oxygen therapy, Reactive oxygen species, Antioxidant defences

1 Introduction

Hyperbaric oxygen therapy (HBOT) is a therapeutic approach where the patient is exposed to 100% oxygen at pressures higher than ambient (1 ATA). This leads to an increased blood oxygen level, which than can penetrate to ischemic areas more deeply than under normobaric conditions [1]. Normally 97% of the oxygen transported from the lungs to the tissues is carried in chemical combination with hemoglobin or red blood cells, and the remaining 3% in a dissolved state in plasma. Under hyperbaric conditions, it is possible to dissolve sufficient oxygen, i.e., 6 vol% in plasma, to meet the usual requirements of the body [2].

HBOT is a standard therapy for decompression sickness, gas embolism and CO poisoning [3, 4]. HBOT is also effective for gas gangrene, anaerobic infection, diabetic foot, Burger's disease and other oxygen-deficient conditions [5, 6]. In addition, HBOT has been proved effective in the healing of chronic wound, such as radiation-induced soft tissue necrosis [7-10]. Meanwhile, many studies reported the therapeutic or preventive effect of HBOT in various kinds of inflammatory or immune-mediated diseases, such as systemic lupus erythematosus [11, 12], atherosclerosis [13], collagen-induced arthritis [14], Crohn's disease [15], ulcerative colitis [16, 17] and atopic dermatitis [18], although these diseases are not included in the current indication of HBOT [19, 20].

Although there is large body evidence that HBOT is useful as a therapy, there is also data indicating that the use of hyperbaric oxygen can have serious side effects. The main concern in HBOT is oxidative stress and/or oxygen toxicity that can affect multiple organs [1]. The severity of oxygen poisoning increases progressively with increase of the inspired partial pressure of oxygen and with greater duration of exposure [21].

2 Free radicals and other reactive species

Increased concentrations of free radical intermediates during exposure to hyperoxia provided a biochemical basis for oxygen toxicity of HBOT [21].

Oxygen radicals represent the most important class of radical species generated in living systems. Reactive oxygen species (ROS) is a collective term that includes both oxygen radicals and certain non-radicals that are oxidizing agents and/or are easily converted into radicals. All

oxygen toxic species are ROS, but not all ROS are oxygen radicals. They can be produced from both endogenous and exogenous substances. ROS play a dual role in biological systems, since they can be either harmful or beneficial to living systems [22]. As ROS are highly reactive and interact with many bio-molecules, they are likely to destroy biological structures, promoting cellular damage and tissue destruction [19]. Therefore, an excessive generation of highly reactive oxidants results in tissue damage, called as oxidative stress [23]. In contrast, many recent evidences are accumulating on the protective role of ROS in immune-mediated diseases [19].

In HBOT conditions activated neutrophils can release into the extracellular environment a variety of reactive species including superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, and peroxynitrite. Free radical damage can occur as lipid peroxidation, amino acid oxidation, protein strand scission, and various cross-linking reactions among lipids and proteins. Peroxidation of membrane unsaturated fatty acids, structural protein oxidation, and inactivation of membrane-bound enzymes can cause the loss of secretory and other important membrane functions by increasing membrane permeability and reducing transmembrane ion gradients [21].

Sharing many biophysical characteristics with oxygen, nitric oxide ($\cdot\text{NO}$) is another physiologic gas that provides an important source of free radicals. At a rate that is nearly diffusion limited, $\cdot\text{NO}$ reacts with superoxide to produce the powerful oxidant peroxynitrite. Much of the peroxynitrite formed *in vivo* reacts rapidly with carbon dioxide to produce a nitrocarbonate intermediate that is an efficient nitrating agent. This reaction also produces intermediates that can affect other tissues in secondary reactions [21].

3 Antioxidant defences

Antioxidant defences have been characterized as a multilayered system that evolved to counteract the adverse effects triggered by the univalent reduction of molecular oxygen. A first line of defense in this system involves the action of enzymes, such as cytochrome oxidase, that can reduce molecular oxygen to water without producing reactive intermediates, thereby avoiding the univalent pathway and reducing the pool of active radicals that must be opposed by other means. Metalloenzymes known as SODs constitute a second line of defense by catalyzing the dismutation of superoxide anion to form hydrogen peroxide. A third line of antioxidant defense is provided by enzymes, such as catalase and glutathione peroxidase, that catalyze the removal of hydrogen peroxide produced either indirectly by superoxide anion dismutation or directly by reoxidation of reduced flavoenzymes. Biologic antioxidants such as vitamin E act as a fourth line of defense by reacting rapidly with chain propagating fatty acid radicals to form a stable α -tocopherol radical and terminate the chain reaction. Reversal of oxidant damage by reactivation of oxidized enzymes and reduction of oxidized tissue components constitutes a fifth line of defense that appears to be provided mainly by interactions with reduced glutathione, producing oxidized glutathione as a by-product. Concurrent activation of the pentose shunt pathway of glucose metabolism supplies the nicotinamide adenine dinucleotide phosphate that is required to regenerate reduced glutathione [21, 24, 1].

Heme oxygenase-1, also known as heat shock protein 32, is highly induced by oxidant stress and has been proposed for a possible protective role against oxidant-induced lung injury. Potential mechanisms for an antioxidant action include catalysis of the oxidative degradation of heme, which can function as a cellular pro-oxidant, and production of bilirubin as an end product that has antioxidant properties. Increased levels of heme-oxygenase-1 were measured in lymphocytes obtained from healthy humans 24 hours after breathing O_2 at 2.5ATA (252 kPa) for 60 minutes on a 20-minute O_2 /5-minute air intermittent schedule. Reversible breakage of DNA strands found in lymphocytes after a single exposure to this profile did not occur after the second or subsequent exposures. In addition, lymphocytes isolated from blood obtained 24

hours after the initial exposure were resistant to DNA damage by hydrogen peroxide *in vitro*. In a related investigation, synthesis of heat shock protein 70 was also induced in lymphocytes by a single 3 x 20-minute O₂ exposure at 2.5 ATA (252 kPa), whereas red blood cell concentrations of SOD, catalase, and glutathione peroxidase were not altered. The principle that the heat shock proteins can provide cross-protection against oxidant injury is supported by the observation that hyperthermic preconditioning of cultured human umbilical vein endothelial cells significantly reduced the cellular damage caused by subsequent exposure to hydrogen peroxide. In addition, heat acclimation of rats before O₂ exposure at 6.0 ATA (606 kPa) doubled the period of latency before the onset of electroencephalographic spikes in association with increased brain levels of heat shock protein 72. During a 4-week period of deacclimation to heat, reversal of the gain in seizure latency correlated directly with decreasing levels of heat shock protein 72 [21].

Furthermore, there is increasing evidence about the ability of HBO to induce cellular protection in a similar manner with other protective oxidative stress mechanisms. It was shown that an initial HBO treatment of human subjects leads to the induction of adaptive response that protects cells against the induction of DNA damage by a second HBO treatment. It is also evident that ROS generated by HBO triggers the upregulation of antioxidant enzyme activities, thereby induces tolerance against ischemia in the tissues [23].

4 EXAMPLES OF PROPOSED MECHANISMS OF ACTION

Autism

Numerous studies document oxidative stress and inflammation in individuals with autism [25-27]. There exist concerns that HBOT might increase oxidative stress via the production of reactive oxygen species (ROS). These concerns are relevant because some individuals with autism express evidence of increased oxidative stress including lower serum glutathione levels, and decreased activities of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase, and catalase [25]. Some autistic individuals also demonstrate evidence of increased lipid peroxidation. Rossignol et al. [28] studied the oxidative stress and inflammation in children with autism, which underwent 40 hyperbaric sessions of 45 minutes duration each at 1.5 ATA and 100% oxygen, or at 1.3 ATA and 24% oxygen because a review of the literature indicates that oxidative stress can occur with HBOT but appears to be less of a concern at hyperbaric pressures under 2.0 ATA. They measured concentrations of C-reactive protein (CRP) and markers of oxidative stress, including plasma oxidized glutathione (GSSG), which were assessed by fasting blood draws collected before and after the 40 treatments. HBOT did not appreciably worsen oxidative stress and significantly decreased inflammation as measured by CRP levels. The proposed explanation can be the fact, that the long-term and repeated administration of HBOT below 2.0 ATA can actually decrease oxidative stress by reducing lipid peroxidation, and by up-regulating the activity of antioxidant enzymes including SOD, glutathione peroxidase, and catalase. However, the effects of HBOT on oxidative stress in autistic individuals are still unknown [28].

Ischemic wounds

HBOT accelerates the healing of chronic wounds and is now primarily used as an adjunctive therapy in managing selected problem wound healing [29]. Despite multiple inciting etiologies, the final common pathobiology of chronic wounds includes oxidative stress caused by high levels of reactive oxygen and nitrogen species (ROS/RNS). In ischemic wounds, neutrophils and macrophages are the major source of ROS/RNS, with gp91-phox (Nox2) in phagocytes being the primary source of SA and inducible nitric oxide synthase (iNOS) producing high levels of NO. Unless removed by SOD, SA rapidly combines with NO to form peroxynitrite, a

powerful oxidant and inducer of cell death, lipid peroxidation, protein nitration, and oxidation. It is understood that ROS signaling is fundamental to HBOT but the exact manner in which HBOT modulates ROS signaling pathways in the wound and protects ischemic tissue from oxidative stress remains unclear [24].

Zhang et al. [24] investigated whether HBOT modulates ROS regulation in ischemic wound tissue. Using validated ischemic wound model with Spague-Dawley rats, they detected high levels of iNOS, gp91-phox, and 3-nitrotyrosine, indicating high-oxidant stress. HBOT not only increased antioxidant enzyme expression, such as Cu/Zn-SOD, catalase, and glutathione peroxidase, but also significantly decreased pro-oxidant enzyme levels, such as iNOS and gp91-phox, thereby decreasing net oxygen radical production by means of negative feedback. They proposed that the negative feedback regulation can be an important mechanism to maintain the dynamic balance of ROS/RNS production and removal at the tissue level. They proposed that HBO treatment of ischemic wounds transiently increases ROS levels, activating the negative feedback loop that downregulates the inducing enzymes (less production of ROS/RNS) and upregulates the antioxidant enzymes (increased removal of ROS) thereby limiting subsequent higher levels of ROS production. Their results further imply that ROS/RNS-related enzymes, such as iNOS and nicotinamide adenine dinucleotide phosphate oxidase, rather than the ROS itself, could be important therapeutic targets for inhibiting oxidative stress.

Boykin and Baylis [29] documented general somatic and wound nitric oxide (NO) levels during and after HBOT. They reported significantly increased local wound NO levels (by NO_x measurements) after HBOT in patients successfully responding to it. In contrast, HBOT had in their study no effect on systemic NO production or on ADMA and L-Arg levels, suggesting no systemic impact of HBOT on the NO system. These data suggested that enhanced local NO-mediated processes may provide an important mechanism for the improved wound healing and closure observed in patients responding to HBOT.

Brain injury

It has been demonstrated, that ROS, if exceed the capacity of the anti-oxidative defense, lead to oxidative stress and cellular damage after brain trauma. In different brain pathologies, the induction of manganese superoxide dismutase (SOD2) varies and depends on the type of injury, and most data point out the neuroprotective role of SOD2 in brain injury. Parabucki et al. [1] evaluated the effect of HBOT on SOD2 expression pattern after the cortical stab injury in Wistar rats. The pattern of SOD2 expression and cellular localization was analyzed using real-time polymerase chain reaction, western blot and double-label fluorescence immunochemistry. Wistar rats were exposed to the pressure of 2.5 ATA for 60 minutes once a day for consecutive 3 or 10 days. Exposure of rats to HBO for 3 days considerably down-regulated SOD2 protein levels in the injured cortex, while after 10 days of HBOT an up-regulation of SOD2 was observed. HBOT significantly increased mRNA levels for SOD2 at both time points. According to their results, HBOT alters SOD2 protein and mRNA levels after brain injury in a time-dependent manner. The results of their study imply that application of HBOT, via altering SOD2 expression, may attenuate imbalance between oxidants and antioxidants that occurs after brain injury, and in that way contributes to the maintenance of pro-/antioxidant homeostasis.

Duration of HBOT

Xeu et al. [30] evaluated the therapeutic effect and the oxidative stress effect of 9 and 18 hour HBOT protocols on the earliest stage of acute permanent middle cerebral artery occlusion in rats. The level of ROS determined by SOD, malondialdehyde (MDA) and NO in ischemic brain tissue were separately examined at the 18, 48 and 120 hour post-ischemia time points

using spectrophotometry. The SOD and MDA levels of the 9 and 18 hour application of HBOT were remarkably lower than those of control group after both 18 and 48 hours. The levels of NO in both HBOT groups were remarkably higher than that of the control at 18 and 48 hour time points. While the level in 18 hour group was remarkably lower than that of 9 hour group at 18 hour time period, at the 120 hour mark, the NO levels were basically the same in all three groups. They concluded the study with the fact, that the longer duration may aggravate the oxidative stress in ischemic tissue.

5 Conclusion

HBOT has a number of physiological and pharmacological models of action. These properties constitute the rationale for treatment of a number of different conditions. Some indications are relatively well documented in clinical studies, but many are supported only by studies that do not comply with modern criteria for a high level of evidence. When HBOT is used appropriately, however, serious adverse effects are rare, and those that do occur are nearly always reversible. Increasing evidence exists that the protective roles of classical antioxidant enzymes such as SOD, catalase, and glutathione peroxidase may be supplemented by other cellular and molecular responses to oxidative stress in mammalian cells. However, the unwelcome side-effects have often been dependent on treatment parameters - pressure and duration of the treatment. Further investigation to explore their full biological potential may prove to be worthwhile.

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