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REVIEW ARTICLE

Similarities and differences of hyperbaric oxygen and medical ozone applications

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Abstract

Hyperbaric oxygen (HBO) treatment is based on the principle of having the patient breath 100% oxygen in an environment above atmospheric pressure. Ozone (O_3) is a colourless gas with a specific odour and consists of three oxygen atoms. The classical scientific understanding is that the world has become a place suitable for life for aerobic organisms with the increasing oxygen in the atmosphere billions of years ago. The formation of ozone after oxygen has then protected aerobic creatures from harmful rays. We now use these two gases for treatment purposes. It is noteworthy that the oxygen and ozone molecules that are formed by the same atom in different numbers are used for similar medical indications. We will try to emphasize the similarities and differences of HBO and medical ozone applications in this article.

Keywords: *oxygen, hyperbaric oxygen, oxidative stress, ozone, ozone therapy*

Introduction

Scientific data indicates that there was no oxygen when the world came into existence. Oxygen first started to form as a waste product of anaerobic organisms approximately 3 billion years ago and started to increase with synthesis by photosynthetic organisms 1 billion years later [1]. The increasing oxygen created the atmospheric ozone layer as a result of reacting with ultraviolet radiation. The source of the ozone gas is also the oxygen in the atmosphere. Oxygen is essential for all aerobic organisms and contributes to the formation of fundamental organic molecules together with hydrogen, carbon, nitrogen and sulfur [1,2]. It is the most common element in the oceans of our world and the third most common element in the universe after hydrogen and helium. Oxygen has a vital importance due to its role in the energy metabolism of aerobic organisms [3].

As much as oxygen is needed for aerobic life, ozone is essential to a similar degree for the continuity of life on earth with its special functions. Almost 90% of atmospheric oxygen is located in the stratosphere layer, 20–50 km to the earth. The remaining 10% is within the troposphere that forms the first 10–15 km section of the atmosphere [4,5]. The ozone in the stratosphere layer is created from the oxygen molecule with the effect of ultraviolet-C (UV-C) rays and degraded by ultraviolet-B (UV-B) rays. The ozone creation and destruction with different wavelengths of ultraviolet radiation thus creates a continuous cycle [6,7]. Ozone creation from oxygen is a reaction that requires a high amount of energy:

$$3O_2 + 68.4 \text{ Kcal} \rightarrow 2O_3$$

The ozone layer in the stratosphere absorbs almost all the UV-B rays that are dangerous for the organisms on earth [8]. Oxygen supports respiration to continue life while ozone supports life on earth by protecting the world from harmful UV rays.

It is natural for two molecules with such importance for life to be used or at least investigated for the treatment of various disorders. Medical ozone and HBO applications are two treatment types based on oxygen. HBO treatment utilizes the two-atom O_2

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molecule while ozone treatment uses the three-atom O_3 molecule.

Hyperbaric oxygen treatment

HBO administration is a treatment method based on 100% oxygen respiration by the patients under a higher pressure than normal atmospheric pressure in special rooms, called hyperbaric chamber [9]. Historically, the British priest Henshaw has tried to treat patients with pressurized air in a special room he developed in 1662 [10]. The French surgeon Then in 1879, Fontaine constructed an operating room where the pressure could be increased and performed more than 20 surgical procedures there [11]. The scientific aspect of HBO use began to be added to these findings reported with pressurized air application when Priestley discovered oxygen, and all these were brought together in 1775 [12]. However, it was also shown, following some complications, that exposure to a high amount of oxygen had the risk to be toxic [13,14]. It can be said that the start of the use of HBO treatment in its current sense was with the treatment of decompression sickness in the American and British navy from the 1930s. HBO treatment is now commonly used in various fields of medicine.

HBO has two well-defined basic effects: One is the compressive and volume-decreasing effect on the gases in the body by purely mechanical means; the other is increasing the amount of dissolved oxygen in body fluids. These two mechanisms are used to explain the therapeutic effect of HBO treatment [9]. Decompression sickness and air embolism are examples of disorders where HBO is effective by mechanical means. The gas bubbles within the vessel occlude the vessel and disrupt tissue perfusion in these pathological conditions. These bubbles become smaller with the pressure the patient is exposed to during HBO administration and are dissolved [15,16]. A very small percentage of oxygen is normally dissolved in plasma, but oxygen that can meet all the basal requirements of the body can be dissolved in plasma under hyperbaric conditions [17]. The hemoglobin becomes 100% saturated with oxygen during HBO treatment, and the oxygen saturation continues as 100% even in venous blood. The amount of oxygen dissolved in the blood under physiological conditions is 0.32 mL/dL increasing to 6.8 mL/dL breathing pure oxygen under 3 atmospheres of pressure ($3 \times 760 = 2280$ mmHg). The partial oxygen pressure can increase up to 1800 mmHg in the arterial blood at this time (Figure 1) [9,17].

This much of increase in partial oxygen pressure during HBO session is life-saving in the treatment of carbon monoxide (CO) toxicity. Studies on rats have shown that HBO treatment supports survival in CO poisoning and significantly decreases the rate of neurological sequelae [18,19]. CO poisoning is still one of the most important clinical indications of HBO treatment [20,21].

Some secondary effects of HBO treatment have been reported in addition to the 'mechanical' and 'increased oxygen solubility' effects thought to be the basic mechanisms of action. These are increased leukocyte defence capacity, decreased leukocyte adherence to vascular walls, increased fibroblast growth and collagen production, stimulation of antioxidant superoxide dismutase (SOD) enzyme production, preservation of ATP in the cell, increased ostoeblastic activity and increased capillary proliferation [12].

The principles and indications of HBO treatment have been standardized by various institutions. One of the best known of these organizations is the 'Undersea and Hyperbaric Medical Society' (UHMS) that

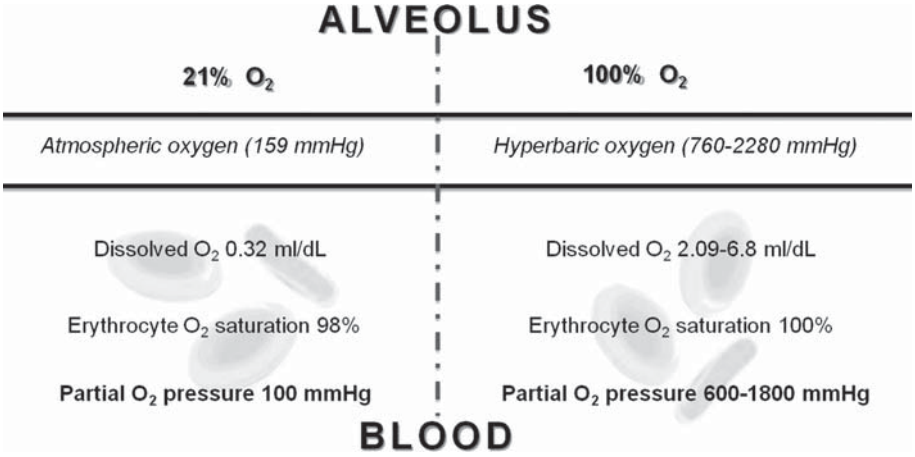

Figure 1. Very little of the oxygen is dissolved in the blood under normal conditions. However, oxygen that can meet the routine needs of the body by itself can dissolve in the plasma under hyperbaric conditions. The figure shows the amount of oxygen dissolved in the blood under normal atmospheric conditions and the partial pressure and hemoglobin oxygen saturation it creates on the left, and how much these figures change by breathing pure oxygen in a hyperbaric environment from normobaric conditions all the way up to 3 atmospheres of pressure on the right.

contributes to the increased knowledge on HBO treatment principles with its congresses, courses, certificate programs and research grants every year. There are currently 13 indications approved by the UHMS with scientific criteria as suitable for HBO treatment (Table I) [9]. These standards were drawn after the evaluation of the data obtained from many research and case control studies [22–29].

Ozone treatment

Ozone (O_3) is a three-atom molecule created as a result of the addition of an oxygen atom to the oxygen molecule. Like oxygen, it is a colourless molecule that is in gas form at room temperature but ozone has a specific sharp odour. The ozone gas was found by the German chemist Christian Friedrich Schönbein in 1839 [30]. Ozone is an effective disinfectant thanks to its strong oxidizing effect. It has, therefore, first been used for disinfection following its discovery. Ozone does not have any features of a radical with its chemical structure but is the third strongest oxidizing substance known following fluorine and persulphate [31].

The first medical use of ozone was by Dr. Albert Wolff who treated gangrene and similar serious wounds of German soldiers during World War I [30,32]. Ozone treatment was then used in occasional cases by physicians and investigators until the 1980s. Scientific studies and case series on the use of ozone for medical purposes began to increase in the literature from the 1980s. Ozone is a reactive gas and actually toxic for living organisms. The lungs and the eyes are the organs most sensitive to the toxic effect of ozone. The irritation of the eyes and the effects on the lung vary according to the concentration, temperature, humidity and duration of exposure. Inhalation of ozone at low concentrations can cause irritation of the throat and result in cough. Inhalation of higher concentrations can cause lung edema due to damage of the bronchial mucosa and pneumocytes [33,34].

Table I. UHMS-approved indications for HBO therapy.

Air and gas embolism
Carbon monoxide intoxication
Clostridial myonecrosis (gas gangrene)
Compartment syndrome and other acute traumatic ischemias
Decompression sickness
Problem wounds
Exceptional blood loss (anemia)
Intracranial abscess
Necrotizing soft tissue infections
Chronic osteomyelitis (refractory)
Skin graft and flaps
Compromised radiation injury
Thermal burns

The principle of ozone treatment is the administration of an oxygen/ozone mixture at a specific ratio (minimum oxygen 95%, maximum ozone 5%) to body cavities or the circulatory system [35]. The method that has become the classical application of ozone treatment was defined by Wolff in 1974. In this method, some (50–270 mL) blood is transferred outside the body and is mixed with an oxygen/ozone mixture in an ozone-resistant bottle for 3–5 min. The blood is then returned to the person (autotransfusion) [36]. This procedure is called major autohemotherapy. It is assumed that the increased partial oxygen pressure in the blood treated with the oxygen/ozone mixture has no therapeutic effect as this relative condition created in a very small amount of blood will become insignificant following dilution with the autotransfusion procedure. The physical and chemical effects of ozone, therefore, seem to play a direct role in this therapeutic effect.

Ozone can be dissolved in water (and plasma) depending on the ambient temperature and pressure and its own concentration, just like other gases (e.g. O_2, CO_2) [37]. Ozone reacts rapidly with biomolecules once dissolved in biological fluids because of its reactive feature in contrast to oxygen. Polyunsaturated fatty acids and thiol compounds containing a sulphydryl group (SH) such as cysteine show high affinity in this reaction [38,39]. Carbohydrates, proteins (and therefore, enzymes), DNA and RNA can also be influenced by this activity depending on the amount of ozone. All these compounds act as electron donors with ozone and are oxidized. Reactive oxygen species (ROS) such as superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2) and hypochloric acid (HClO) are formed as a result (Figure 2) of such oxidation. The most important of these reactions is the oxidation of unsaturated fatty acids due to the high affinity. The reaction formula is as follows:

$$\mathrm{R{-}CH{=}CH{-}R' + O_3 + H_2O \rightarrow R{-}CH{=}O + R'{-}CH{=} + H_2O_2}$$

Two lipid oxidation products (LOP) are formed with each hydrogen peroxide molecule in this reaction [31,38,40–42]. Some well-known LOPs are lipoperoxyl radical, hydroperoxides, malondialdehyde, isoprostan, alcenals and 4-hydroxy-2,3-trans nonenal [43–46]. Lipid oxidation products are able to circulate via the bloodstream through the entire body and are accepted to be responsible for at least some of the therapeutic effects of ozone (Figure 3).

The H_2O_2 formed as a result of the reaction between ozone and biomolecules is accepted to be a second molecule acting as a mediator for ozone treatment effects (Figures 2 and 4) [47–49]. One of the first effects of H_2O_2 is a shift to the right in the hemoglobin-oxygen dissociation curve by increasing the erythrocyte 2,3-diphosphoglycerate level and therefore

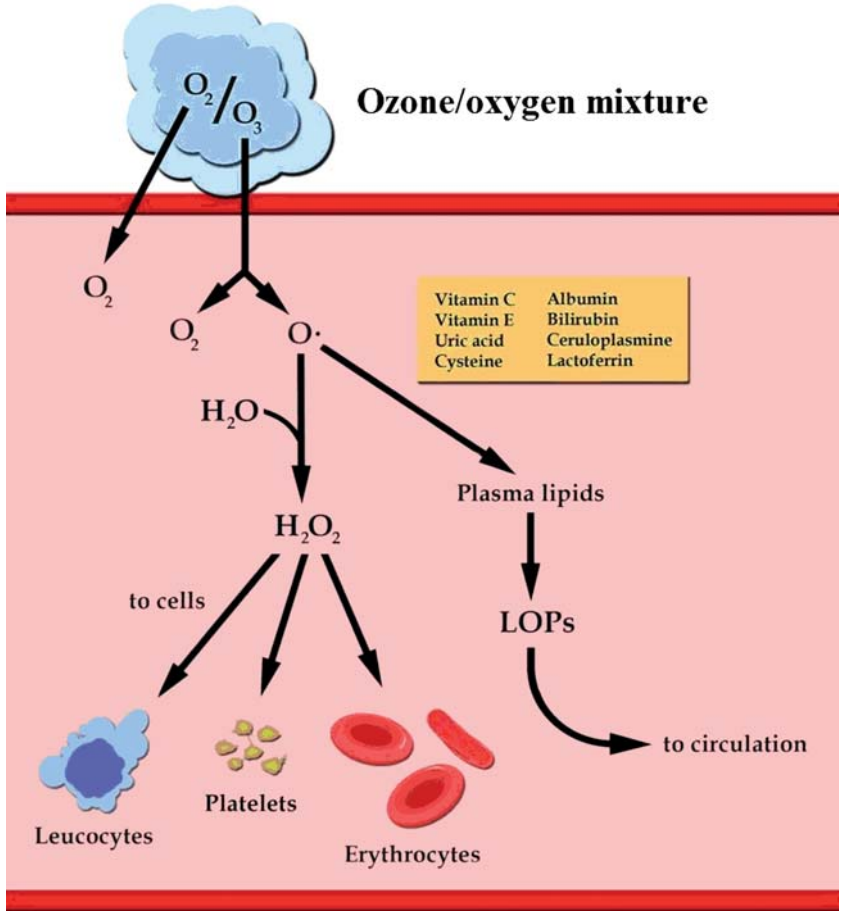

Figure 2. Ozone is rapidly separated into an oxygen molecule and oxygen radicals after the administration of the ozone/oxygen mixture into the blood (autohemotherapy). The antioxidant system of the plasma tries to neutralize the radicals that are created. Once the antioxidant system has been partly saturated, the remaining oxygen radical oxidizes plasma lipids (especially PUFA) and results in the development of H_2O_2. The H_2O_2 and LOPs created act as signal molecules. Hydrogen peroxide has a short half-life (seconds) while LOPs have long half-lives (hours). The oxygen in the ozone/oxygen mixture has no therapeutic effect; it acts as a diluent gas to adjust the ozone concentration.

facilitating oxygen release to the tissues [50]. The H_2O_2 that enters leukocytes and endothelial cells can stimulate the synthesis of various cytokines (e.g. interleukin family, IL; transforming growth factor, TGF; platelet-derived growth factor, PDGF) in these cells [35,48,51–54].

Thanks to these properties, ozone treatment can be used for therapeutic purposes in many different conditions where the immune system plays a role in the pathophysiologal process, and other pathologies that develop on an ischemic and infectious basis such as problematic wounds in general. It has been reported to be effective in a wide range of problems from age-related macular degeneration and simple dental and oral infections to hepatitis [55–58].

Differences between hyperbaric oxygen and ozone treatment

Hyperbaric oxygen treatment is more commonly accepted and in use than ozone treatment at present. This is partly due to the relatively larger number of published experimental and clinical studies with HBO treatment [58–61]. Work by organizations such as the UHMS has also contributed to the institutional acceptance of HBO treatment. For example, although detailed administration conditions of HBO treatment such as pressure, duration, oxygen density and frequency of application were not yet specified in the early 1960s, the FDA approved its clinical application for wound healing because of the positive results reported [62].

A PubMed database search on September 2011 with the 'ozone therapy' key word gave 241 results while 'hyperbaric oxygen' produced 5114; half of those on ozone treatment and a fifth of those on HBO are studies within the last 10 years. These results indicate that there is less data accumulation regarding ozone treatment than HBO treatment in the current medical literature. However, acceptance by scientific journals of articles on ozone treatment, despite their low number, and interest in this field has been increasing.

Trained staff that is aware of the necessary safety precautions are required both for HBO and ozone

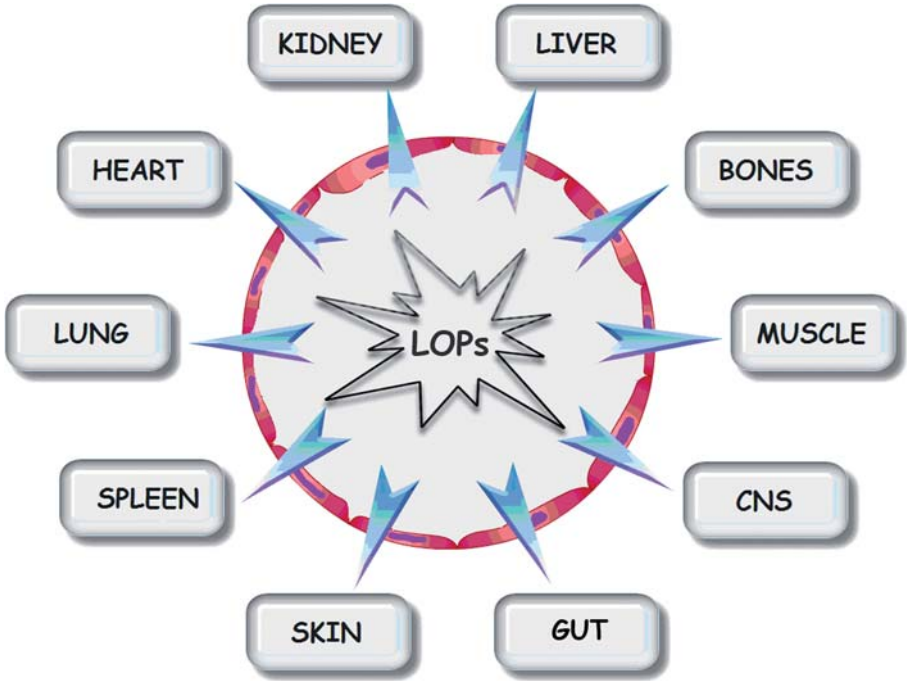

Figure 3. The LOPs created during ozone administration are distributed into the body with their long half-life and act as mediators for the effects of ozone on distant organs. (Modified from Bocci [49]).

treatment. HBO administration requires a pressure-resistant special cabin while ozone treatment requires an ozone generator and application material made of ozone-resistant material (i.e. stainless steel, neutral glass and teflon) [9,31]. HBO treatment is generally applied systemically and has limited local use whereas ozone treatment can be administered in many different ways (e.g. intramuscular, intraarticular, intrapleural, intrarectal, intradiscal and topical) [31]. A session of HBO treatment takes 1–2 hr whereas this figure is 20–30 min for ozone treatment. Both treatment types continue for a long time, for example, 15–20 sessions, but HBO treatment is generally planned as 5 sessions a week with mostly consecutive days while 2–3 sessions a week is adequate for ozone treatment.

Table I presents the UHMS-specified indications for HBO treatment. There is no such institutional study for ozone treatment. In practice, ozone and HBO treatments can be said to overlap for some indications. Wound healing can be accepted as the most

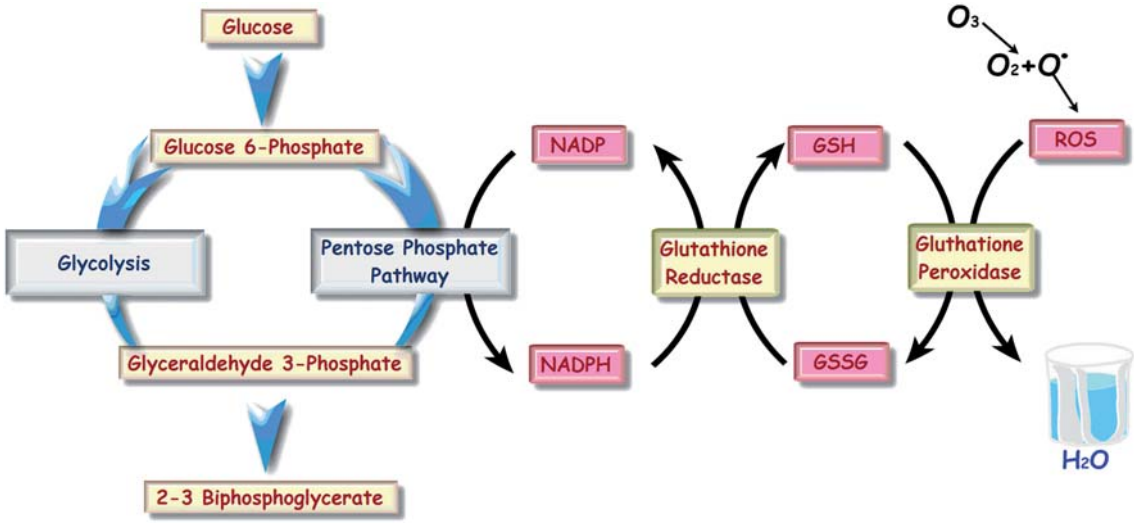

Figure 4. The cellular antioxidant systems are stimulated to sweep the ROS, especially H_2O_2, that increase following ozone autohemotherapy applications. More NADPH is used following the increase of glutathione peroxidase (GSH-Px) enzyme activity, especially in the erythrocyte, and this is replaced via the pentose phosphate pathway. The use of this pathway leads to increased 2,3-bisphophoglycerate. The increased 2,3-bisphophoglycerate shifts the hemoglobin-oxygen dissociation curve to the right. and it, therefore, becomes easier for hemoglobin to release its oxygen to the tissues. Ozone treatment, therefore, contributes to tissue oxygenation as a result [38].

common medical application among these common indications. HBO treatment is used quite commonly in problematic wound healing conditions such as the diabetic foot [63]. When the arterial oxygen pressure is approximately 100 mmHg, the partial oxygen pressure will be 0–10 mmHg at the skin wound center and 60 mmHg at the wound periphery [64]. Wound healing can be disturbed in various conditions accompanied by this natural hypoxic condition of the wound environment. HBO treatment can help by regulating some disturbed repair processes (collagen synthesis, VEGF, etc.) to increase oxygen in the wound environment. HBO treatment supports healing with adequate oxygen support in such cases [63]. Such wounds are also one of the common application areas of ozone treatment [38]. As mentioned above, one of the main effects of ozone treatment is the easier release of oxygen to tissues by increasing the erythrocyte 2,3-diphosphoglycerate level [50]. This may be interpreted as the biochemical support by ozone of tissue oxygenation that is ensured with the help of pressure by HBO.

In addition to its effects on erythrocytes, ozone treatment also improves platelet functions. It has a beneficial effect in patients with ischemia and necrosis by releasing the growth factors in activated thrombocytes. Studies have revealed that ozone applications increase the release of cytokines such as PDGF, TGF-ß1 and IL-8 from platelets [65,66]. Kim et al. [67] have reported a significant acceleration of wound healing in wound tissue administered topical ozone accompanied by increased expression of PDGF, TGF-ß1 and VEGF. Similarly, HBO treatment is known to have a stimulating effect on growth factors in addition to oxygen support [64,68].

The presence of some common indications for HBO and ozone treatment requires the comparative strengths and weaknesses of these methods for specific indications be drawn out. Such a result requires carrying out comparative studies. However, there are no studies comparing HBO and ozone treatment in the literature. The comparative efficacy of HBO and ozone treatment is still being investigated with experimental studies by our team. For example, Uysal et al [69] have shown that ozone treatment decreases necrosis and bacterial translocation more than HBO in rats where necrotizing pancreatitis was created. Another study has demonstrated in a rat ulcerative colitis model that ozone treatment decreases necrosis and inflammation in a more significant manner than HBO [70]. The studies that are yet few in number indicate that ozone treatment is as effective as HBO treatment or perhaps sometimes more effective in pathologies developing on an inflammatory basis. Bocci, with wide accumulation of data on ozone treatment, has published the differences in efficacy in various indications of these two treatment modalities by using his own experiences with ozone and reviewing studies on HBO treatment, in his book (Table II) [71].

Table II. Comparison of the therapeutic efficacy of HBO and ozone therapy according to Bocci [70].

	HBO Therapy	Ozone Therapy
Arterial gas embolism	+++	---
Decompression sickness	+++	---
Severe CO poisoning	+++	---
Severe blood loss-anemia	+++	---
Clostridial myonecrosis (gas gangrene)	+++	++
Compromised skin grafts and flaps	+	+++
Prevention of osteo-radionecrosis	+	+++
Radiation damage	+	+++
Refractory osteomyelitis	+	+++
Necrotizing fasciitis	+	+++
Traumatic ischemic injury	+	+++
Thermal burns	+	+++
Chronic ulcers and failure of wound healing	+	+++
Multiple sclerosis	---	+?
Chronic fatigue syndrome	+	++
HIV-AIDS	+?	+
Senility	+	++

(+ little, ++ modest, +++ good activity, --- no activity)

Conditions such as gas embolism, decompression sickness or CO poisoning seen in the table are examples of indications where the use of HBO is life-saving, and there would be no such expectation from ozone treatment when the mechanism of action is considered. Both treatment forms can be helpful in other conditions and those pathophysiological processes that mainly develop on an inflammatory basis but the lack of controlled case series that compare these two treatment forms is unfortunate.

The side effects and complications of HBO treatment are generally accepted to be rare and insignificant. However, important problems such as central nervous system toxicity characterized by convulsion can also rarely occur. Other adverse affects include dental problems, hypoglycemic seizures, transient myopia and barotrauma that can especially present as pneumothorax due to lung barotrauma. Oxygen itself plays an important role in the potential toxicity risk of HBO. Lavoisier and Seguin were the first to report that oxygen has various toxic effects in 1789 [15].

HBO applications under 1.5–2 atmosphere pressure for up to 1-hour duration are generally accepted to be a safe dose and are well tolerated by both experimental animals and humans. [72]. However, therapeutic HBO procedures are usually above this safe range of 2 atmosphere and 1 hr. Many studies have, therefore, reported an increase in free radical formation and related lipid peroxidation with the application conditions used [73–77]. The number of conditions where HBO application is contra-indicated is relatively low when compared with many other treatment methods.

Table III. Contraindications of hyperbaric oxygen therapy [78].

Absolute contraindications	Untreated pneumothorax
	Doxorubucin treatment
	Bleomycin treatment
	Disulfram treatment
	Cisplatin treatment
	Mafenide acetate treatment
Relative contraindications	Upper respiratory infections
	Middle ear barotrauma
	Emphysema with CO_2 retention
	High fever
	Spontaneous pneumothorax
	History of chest surgery
	Epilepsy

However, pre-existing conditions and concurrent treatment may create contraindications for HBO treatment (Table III) [78].

Side effects of ozone treatment are almost non-existent. All the side effects reported so far are local complications due to application errors [79]. Conditions where ozone treatment administration could cause problems are glucose-6-phosphate dehydrogenase enzyme deficiency (favizm), pregnancy (especially the early stage), angiotensin converting enzyme (ACE) inhibiting treatment, uncontrolled hyperthyroidism, bleeding disorder, uncontrolled cardiovascular disease and asthma patients who react to ozone [79,80].

Oxidative effect of HBO and ozone treatment

Free radicals can form due to many factors that are internal or external (intoxication, hemorrhage, ischemia, radioactivity, allergy, air pollution, cigarette smoke, carbon monoxide, insecticides, infection, aging, stress, etc.) to the metabolic processes in living organisms that use aerobic respiration. Free radicals are mostly oxygen-derived but can also be derived from carbon or nitrogen. Those that are derived from oxygen are also named free oxygen radicals or reactive oxygen species (ROS). These free radicals are chemical structures that contain unpaired electrons in their outer orbits. The presence of an unpaired electron greatly increases the reactivity of a chemical molecule. Radicals are therefore compounds with a very short half-life and high reactivity [81–83].

Aerobic organisms have developed antioxidant systems to escape the toxic effects of free radicals. With a general classification, these are separated into two groups; non-enzymatic (glutathione, uric acid, ascorbic acid, proteins and especially albumin, non-protein thiols and bilirubin) and enzymatic antioxidants. The main antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT) and elements of the glutathione (GSH) cycle consisting of glutathione peroxidase (GSH-Px), glutathione transferase (GST) and glutathione reductase (GR) [40].

ROS formation is increased due to the increased oxygen in many tissues during HBO treatment, and this can cause oxidative stress. This is normal in an environment where oxygen is used so intensely. The reason for this increase is the increased electron transfer to oxygen in hyperoxic conditions where radical creation occurs physiologically in the mitochondrial oxidative phosphorylation chain (Figure 5) [84–86].

The oxygen molecule can easily show the characteristics of a radical due to the unpaired electrons in its atomic structure [83]. The knowledge that oxidative stress is the main pathological factor underlying disorders and its role in stress damage

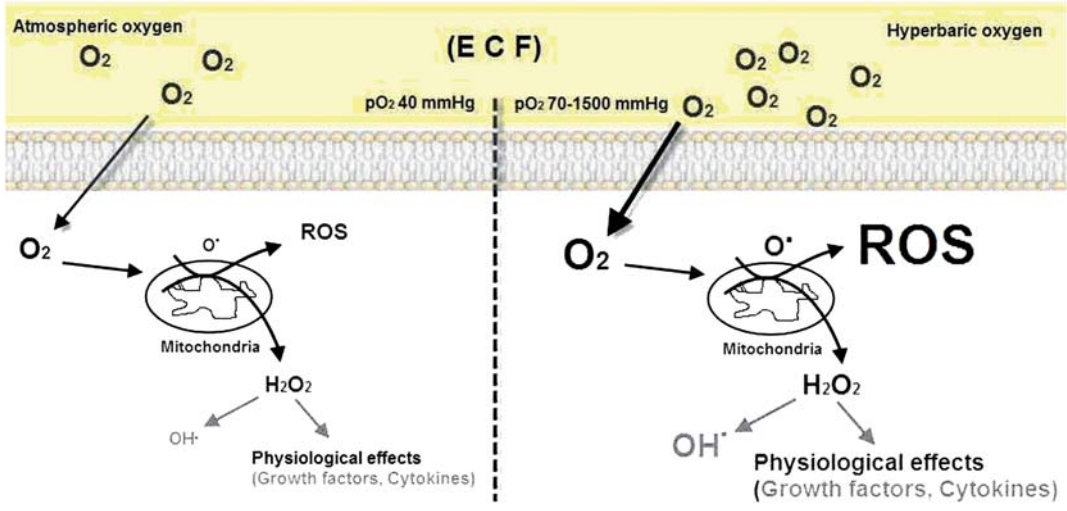

Figure 5. Under normal conditions, 1–2% of the oxygen used in the electron transport chain ends in superoxide radical creation. The tissue partial oxygen pressure has been shown to increase to levels up to 1500 mmHg during HBO treatment [87]. The increased partial oxygen pressure increases ROS production under hyperbaric conditions while H_2O_2 that is responsible for part of HBO's therapeutic effect also increases.

to the cell has made HBO treatment suspicious for many years. Although hyperbaric oxygen is known to cause oxidative stress, it has not been possible to elucidate the degree of this oxidative stress, and how harmful it is. The oxidative stress that arises seems to be mostly directly correlated with the application pressure [74] and duration [87,88] of the HBO treatment. There are many studies aiming to decrease the oxidative stress during HBO treatment or to show the mechanism of increase [76,77,89]. Routine administration of antioxidants before treatment has been suggested for HBO clinics, but this has not been included in current treatment protocols. However, administering antioxidant vitamin E + C prophylaxis to patients about to undergo HBO treatment is suggested in many clinics in the routine approach.

In contrast to the worries about oxidative stress created by HBO treatment, ozone has entered the market as a direct oxidizing product, and it has, therefore, been used first for disinfection. A therapeutic effect has, therefore, been accepted from such a reactive and toxic gas. The therapeutic effect of ozone is currently explained via ROS that have been held responsible for the adverse effects of HBO treatment at first [47,90]. The oxidative stress created directly as a result of ozone's reactivity during ozone application is the result of the increase in ROS leakage from the oxidative phosphorylation chain due to hyperoxia in HBO treatment. This similarity indicates that ROS may play a role in the therapeutic effect of both treatments. The focus was always on the role of oxidative stress on cellular damage and its effects of the pathophysiological processes underlying the disorders until recently. Investigators have carried out many studies on explaining the increased oxidative stress mechanisms and effects in various pathological processes [83,91–94]. It was later observed that oxidative stress could also act as mediator for beneficial effects [95] and studies have shown that oxidation/reduction (redox) reactions play a role in biological mechanisms and especially intracellular communication. It is now known that reactive molecules and oxidation products play important physiological roles in the cell in low (physiological) concentrations [96–98].

Various cytokines and growth factors play an important role in most cellular and intracellular communication; some examples are IL-1ß, IL-6, IL-3, tumour necrosis factor-α (TNF-α), angiotensin II, PDGF, nerve growth factor (NGF), TGF-ß1 and fibroblast growth factor-2 (FGF-2). It is well known that ROS act as a sort of secondary transmitter in the appearance of these communication molecule's physiological effects [99]. Hyperbaric oxygen treatment was reported to use ROS for its beneficial actions [100,101]; the same mechanisms can be logically adapted to ozone therapy.

The antioxidant system in response to HBO and ozone treatment

There are interesting similarities in the way the antioxidant system of the body reacts to HBO and ozone treatment [102]. The organism responds to the oxidative stress by adaptation when both HBO and ozone treatment is continued. A study with Jurkat T cells has revealed that the antioxidant enzymes increase in direct relation to the ozone dosage during ozone administration [103]. Hernandez et al. [104] have demonstrated that long-term ozone treatment increased erythrocyte GSH-Px and glucose-6-phosphate dehydrogenase enzyme activities in their study on patients who had suffered myocardial infarction. It has also been stated in other studies where ozone treatment was used that the beneficial effect of ozone may be due to the increase in antioxidant enzymes [105–107].

Similar to ozone treatment, repeated HBO administrations are known to stimulate the endogenous antioxidant defence system of the body and increase antioxidant enzyme systems [74]. Dennog et al. [108] have observed that the oxidative DNA damage seen in the first session in lymphocytes isolated from blood samples obtained from volunteers who had undergone HBO treatment was not seen again in the later sessions. These authors also reported that the DNA chain breaks seen after the first HBO session were rapidly repaired and such an effect was not seen after the first 24 hours [109]. Other studies have also stated that some adaptive mechanism becomes active following the first exposure to HBO and that this leads to adaptation to oxidative stress and resultant protection from the oxidative effects of later HBO applications [110,111]. Rothfuss and Speit have shown in their cell culture studies that this activating effect of HBO on these adaptive protective mechanisms can appear not only following exposure to HBO but also to various oxidizing agents [112].

This information creates another common factor of HBO and ozone treatment. Adaptation can develop against the oxidizing effect of both HBO and ozone treatment. This adaptation is not only against continuing HBO and ozone application but also protects the organism against other harmful effects and possibly plays a role in the healing effect seen in several pathophysiological processes.

An interesting common molecule: heme oxygenase-1

Heme oxygenase-1 (HO-1), also known as the heat shock protein 32 (Hsp32), is a microsomal enzyme that has its function in the dissociation pathway of the heme ring. This enzyme can be stimulated by increased oxidative stress, proinflammatory cytokines and nitric oxide (NO). Its main function is

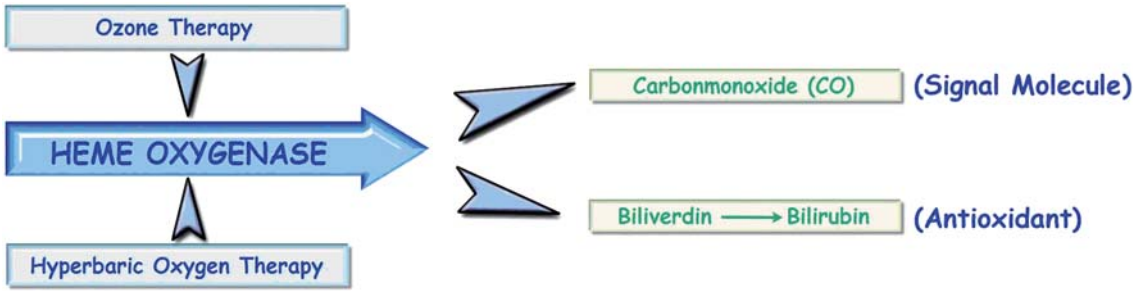

Figure 6. HO-1 is an important enzyme that increases in many pathological states and with oxidative stress. This increase is a protective response of the organism. HO-1 breaks down the heme molecules that appear following damage into biliverdin, iron and CO. Biliverdin is converted to bilirubin with the biliverdin reductase enzyme. Both CO and bilirubin are molecules with important physiological roles.

breaking down the heme molecule to biliverdin and carbon monoxide (CO) [113]. The multidimensional protective effect of this enzyme in many pathological conditions has been emphasized in recent years. The CO that appears following the functioning of this enzyme can act both as an antioxidant and a neurotransmitter and also a vasodilator [114,115]. The strong antioxidant effect of the bilirubin that also appears following the functioning of this enzyme has been known for a long time. HO-1 itself has been used in many pathological conditions and beneficial effects have been reported [116–118].

Bocci et al. [119] have shown that serums obtained from blood administered an ozone/oxygen mixture at various doses increase the HO-1 enzyme in human endothelium cell cultures. The most effective HO-1 increase was in the 20–80 μg/mL range that is also accepted to be the therapeutic dose range of ozone in this study. HO-1 was accompanied by an increase in Hsp70, another thermal shock protein, in the same study. Hyperbaric oxygen was also shown to increase HO-1 in healthy volunteers [120] (Figure 6). This HO-1 increase is thought to play a role in the oxidative stress adaptation of antioxidant systems as explained above [111,121]. Both ozone and HBO studies have found that HO-1 enzyme increase is accompanied by H_2O_2; it was postulated that the HO-1 enzyme increase is due to H_2O_2.

Conclusions

We tried to point out the similarities and differences between HBO and ozone treatments in light of the current information. It is interesting that the findings that may indicate similar aspects at the molecular level of these two treatments seem to be very different at first glance. The most prominent common aspect seems to be the oxidative stress they trigger. Producing a therapeutic effect through increased levels of oxidative stress also seems to fall contrary to the classical medical approach. Oxidative stress is still blamed for a considerable number of pathologies today. Aging, even cancer is thought to be associated with oxidative stress. Oxidative molecules are also known to play a role in the physiological process, however. This emphasizes ROS and the 'controlled' oxidative stress they cause and the associated H_2O_2 and HO-1 increase as important mediators of the therapeutic effects of both HBO and ozone application. It is obvious that this oxidative effect of HBO and ozone application can be safely made use of in treatment with proper dose adjustment, as with any pharmacological agent when avoidance of side effects is desired.

Despite their common aspects, both treatment types also have unique features. The differences of HBO and ozone treatments may create advantages, disadvantages and maybe complementary features. However, there are almost no studies in the literature on this subject. More intensive comparison of HBO and ozone treatment with both experimental and clinical studies is a major need. This will provide important data such as which treatment modality is more effective in similar indications and what results can be obtained with a combination of these two treatments.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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