



## Review

# Hyperbaric oxygen therapy: Antimicrobial mechanisms and clinical application for infections

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## ABSTRACT

Hyperbaric oxygen therapy (HBOT) is a treatment procedure that involves breathing 100% O<sub>2</sub> for a certain time and under a certain pressure. HBOT is commonly administrated as a primary or alternative therapy for different diseases such as infections. In this paper, we reviewed the general aspect of HBOT procedures, the mechanisms of antimicrobial effects and the application in the treatment of infections. Parts of the antimicrobial effects of HBOT are believed to result of reactive from the formation of reactive oxygen species (ROS). It is also said that HBOT enhances the antimicrobial effects of the immune system and has an additive or synergistic effect with certain antimicrobial agents. HBOT has been described as a useful procedure for different infections, particularly in deep and chronic infections such as necrotizing fasciitis, osteomyelitis, chronic soft tissue infections, and infective endocarditis. The anti-inflammation property of HBOT has demonstrated that it may play a significant role in decreasing tissue damage and infection expansion. Patients treated by HBOT need careful pre-examination and monitoring. If safety standards are strictly tracked, HBOT can be considered a suitable procedure with an apt rate of complication.

## 1. Introduction

Antibiotics have decreased the morbidity and mortality rate of microbial infections and are considered a main advancement of modern medicine [1]. Antibiotics have had a remarkable influence on increasing the life span of patients by altering the clinical outcome of bacterial infections. They also play a critical role in the achievement of some advanced therapeutic procedures such as surgery, implant placement, transplantation and chemotherapy. Unfortunately, antibiotic efficacy has decreased over time due to the devolvement of antibiotic resistant pathogens. The resistance phenomenon has been reported in all classes of antibiotics as a result of mutations in microorganisms. Selection pressure from antimicrobial agents offers a competitive circumstance that results in an increase of mutated resistant strains. Recently, the discovery of antibiotics is not easily predicted, and so far resistance has disseminated to all antimicrobial agents, regardless of the chemical features or molecular mechanisms of the antibiotics [2–4]. For better management of the global antimicrobial resistance challenge, a reduction in the amount of antibiotic usage for choice pressure

diminution, proficient infection control policy in order to decrease the spread of resistant pathogens, and alternative treatments, is direly needed [4,5]. Hyperbaric oxygen therapy (HBOT) is a treatment procedure that includes the breathing in of 100% O<sub>2</sub> for a set period of time and under a certain pressure. HBOT has been described as either a primary or alternative technique for the treatment of infections. Regarding the increase in antibiotic resistance frequency, the use of HBOT may be effective in the treatment of acute infections caused by antibiotic resistant pathogens [6]. The aim of this study is to give an overview of HBOT antibacterial mechanisms and application complications for the treatment of infections.

## 2. HBOT procedure

HBOT is a technique in which a patient is exposed to 100% oxygen (O<sub>2</sub>) for a determined period of time and a certain pressure, which is higher than atmospheric pressure, in a special monoplace or multiplace chamber. O<sub>2</sub> pressure for HBOT should be at least 1.4 atmosphere absolute (ATA) or higher. In a monoplace chamber, an individual patient

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breathes in directly pressurized 100% O<sub>2</sub>. In the multiplace chambers more than one patient, breath pressurized 100% O<sub>2</sub> indirectly by a head hood, mask or endotracheal tube. HBOT should not be confused with tropical O<sub>2</sub> therapy. Tropical O<sub>2</sub> therapy is local delivery of O<sub>2</sub> under pressure to a particular part of the body [7]. Delivering O<sub>2</sub> to the lungs leads to an increased level of circulation and tissue O<sub>2</sub> during HBOT. HBOT is commonly administrated as a primary or alternative therapy of inflammation, carbon monoxide (CO) poisoning, chronic wounds, ischemia and infections [8].

### 3. Clinical application of HBOT in infections

Currently, there is sufficient evidence to suggest that HBOT offers valuable advantages, either alone or as an adjunct treatment, for patients with infectious diseases. It has been demonstrated that HBOT considerably stimulates the levels of O<sub>2</sub> concentration in blood, which is typically very low at normal atmospheric condition but is enough to provide the primary need for normal tissue. This finding shows the basic mechanism behind the administration of HBOT in patients suffering from CO toxicity and acute anemia [9,10]. During the HBOT procedure, the O<sub>2</sub> pressure in arterial blood can increase to 2000 mmHg, and the high blood-to-tissue oxygen pressure gradient increases the tissue O<sub>2</sub> pressure to 500 mmHg [11,12]. This effect is considered to be valuable for the healing of inflammatory and micro-circulatory disorders in ischemic circumstances and the compartment syndrome. HBOT also offers anti-edema effects by vasoconstriction, decreases leucocyte chemotaxis and adhesion, attenuates ischemic-reperfusion damage and suppresses the formation of inflammatory mediators. Moreover, the effects of HBOT on the immune system dependent conditions have been extensively studied. For instance, HBOT has been revealed to inhibit the autoimmune syndrome and the immune reaction in antigens, and has also been described as decreasing circulating lymphocytes and leukocytes and adjust immunology in order to maintain the durability of an allograft [13]. HBOT is reported to improve chronic skin damage healing by inducing angiogenesis. The mechanisms of the beneficial effects of HBOT on vascular endothelium, as the responsible tissue for angiogenesis, has been the subject of several studies. HBOT has been described to induce partial high tensions of O<sub>2</sub> in circulating plasma. This stimulates O<sub>2</sub> dependent collagen matrix formation, which is an essential phase in wound healing [14]. HBOT may be a useful approach in the treatment of some infections especially in deep and recalcitrant infections such as necrotizing fasciitis, osteomyelitis and chronic soft tissue infections and infective endocarditis [15,16]. The benefit of HBOT for sepsis, urinary tract infections and meningitis are not well known. The most frequent clinical application of HBOT is for several skin soft tissue infection and osteomyelitis infections which are associated with hypoxia, caused by anaerobic and infections due antibiotic resistant bacteria [17,18]. Table 1 is an overview of some clinical studies investigating the application of HBOT for different infections. The results of *in vitro* or animal models is not included in the table.

#### 3.1. Diabetic foot infections

Foot ulcers are frequent complication in diabetic individuals with the incidence as high as 25%. Infections are a common (40%–80%) and costly problem of these ulcers that can increase the risk of morbidity and mortality [19,20]. Diabetic foot infections (DFIs) are commonly polymicrobial infections and both obligate and facultative anaerobic bacterial pathogens were isolated from these infections [19,21]. Several factors can have an effect on wound healing in diabetic patients, including deficiency of fibroblastic function, collagen formation, cellular immune mechanisms, and phagocyte function. Impaired cutaneous oxygenation has been reported by many studies as being the strongest risk factor resulting in amputation of DFIs. Low O<sub>2</sub> pressure and hypoxia have unfavorable effect on the innate function of leukocytes and

fibroblasts during inflammatory response and healing [16,22]. HBOT is one of the current options for the treatment of DFIs. The application of HBOT was reported to have considerably increased the frequency of healing in foot ulcers, of diabetic individuals, and decreased the need of amputations and debridement that require surgical equipment. HBOT also decreased the necessity of other expensive and technically more involved surgical procedures, such as skin flaps and grafts. HBOT is a beneficial method for the treatment of non-healing diabetic foot ulcers, because the low cost of HBOT compared to that of surgical procedures, commonly only accessible in a clinical setting, limited complication and toxicity [23]. Chen et al reported that more than 10 sessions of HBOT increased the wound healing rate by 78.3% in diabetic patients [16].

#### 3.2. Necrotizing soft tissue infections

Necrotizing soft tissue infections (NSTIs) are commonly polymicrobial infections caused by the synergistic occurrence of different aerobic or anaerobic, in the most cases gas producing, bacterial pathogens. NSTI development is often fulminant, and although it is uncommon, it can cause a high mortality rate [24,25]. Quick and appropriate diagnosis and treatment can possibly increase the chance of a favorable result [26]. HBOT has been recommended as an adjunctive method in the treatment of NSTIs. However, the use of HBOT in the treatment of NSTIs is controversial; because no prospective controlled study has been available for this life-threatening disorder. Such assessment would be difficult to perform because of the relatively low frequency of disease [27]. Nevertheless, HBOT could be associated with increased survival and organ salvage and should be considered in the case of NSTIs [28]. A retrospective study indicated that in spite of the higher cost and longer hospitalization duration, HBOT significantly reduced the mortality rate of patients with NSTI [27].

#### 3.3. Surgical site infections

Surgical Site Infections (SSIs) are infections affecting either the incision or soft tissue at a surgical site. SSIs are further classified in terms of anatomic location. Despite progress in the infectious control procedure, for example sterilization technique and the use of antimicrobial agents for prophylaxis and advancement of surgical techniques, SSIs have continued to be a postoperative problem. SSIs can increase the costs of hospitalization and prolong the duration of the hospital stay. In addition, they can increase the risk of morbidity and decrease the life quality in patients after surgical procedure [29,30]. SSIs have mono- or polymicrobial etiology caused by both anaerobic and aerobic bacteria [31]. The effects of HBOT on the prevention of deep SSIs in neuromuscular scoliosis operation were studied in a retrospective survey. Pre-surgery HBOT may decrease the incidence of SSIs and promote wound healing in neuromuscular scoliosis operations. HBOT is a harmless and beneficial supplement for the prevention of deep SSIs in complicated spine abnormalities in high risk neuromuscular cases [32]. Partial pressures O<sub>2</sub> and wound tissue O<sub>2</sub> levels have been reported to be associated with oxidative killing of pathogens and have been indicated to prevent SSIs [33]. Decreased local blood and O<sub>2</sub> levels are the factors that stimulate the development of SSIs [32]. In addition to other infection control strategies, HBOT has been recommended for the reduction of SSI incidence, particularly during clean-contaminated operation such as colorectal surgery [33].

#### 3.4. Thermal burns

Burns are injuries of skin and subcutaneous tissues of organs as a result of high temperature, electricity, chemicals or radiation [34]. Severe burns are associated with high rate morbidity and mortality in patients [35]. HBOT increases the levels of O<sub>2</sub> in burned tissues. There are controversial reports of HBOT efficiency in the treatment of burns in animal and clinical studies [35–38]. Brannen et al., in a randomized

**Table 1**  
Overview of some clinical studies investigating the application of HBOT for different infections.

Infections	Study population	Treatment sessions	Pressure (ATA)	Exposure Time (min)	Main findings	Ref
Burns	53	based on outcome	2.5	90	All the patients survived	[35]
Burn	40	10	2.5	80	Faster healing, shorter hospitalization	[34]
Brain abscess	41	20 (range, 4–52)	2.5–2.8	25–	Less treatment failures, improved outcome	[111]
SSIs	42	30	2.4	90	Reduce the rate of post-surgical deep infections in complex spine deformity	[32]
SSIs	32	based on outcome	2-3	90	Valuable addition to the armamentarium available to physicians for treating postoperative organ/space sternal SSI	[112]
SSIs	6	based on outcome (28–106)	2.5–2.8	75	Adjuvant treatment to the standard therapy of early postoperative deep infections	[113]
NSTI	48	based on outcome	3	90	Not reduce the mortality rate, number of debridement, hospital duration, or duration of antibiotic use	[114]
NSTI	44	based on outcome	2.8	60	Improved survival and limb salvage	[28]
NSTI	32	based on outcome	2.8	45	Adjuvant treatment, consideration of HBOT should never delay operative therapy	[115]
NSTI	37	based on outcome	2.5	45	The results of this study cast doubt on the suggested advantage of HBO in reducing patient mortality and morbidity when used as adjuvant therapy for NF.	[116]
DFIs	100	based on outcome (20 to 30)	2–3	90	Useful adjunct in the treatment of nonhealing DFIs	[23]
DFIs	42	Group1: < 10 Group 2: > 10	2.5	120	The amputation rate was decreased	[16]
DFIs	94	40	2.5	85	Facilitates healing of chronic DFIs	[117]
DFIs	35	38 ± 8	2.2–2.5	90	Effective in decreasing amputations	[118]
DFIs	28	20	2.5	90	Effective in accelerating the healing rate of nonischemic chronic DFIs	[119]
DFIs	36	20	2.5	90	Healing response in chronic DFIs	[120]
DFIs	38	40–60	2.5	90	Accelerate the rate of healing, reduce the need for amputation	[121]
DFIs	18	30	2.4	90	Valuable adjunct when reconstructive surgery is not possible	[122]
Osteomyelitis	6	30	2.0–2.4	30	Effective following failure of primary therapy of osteomyelitis	[47]
Osteomyelitis	14	30	2.5	120	Effective and safe for chronic refractory osteomyelitis	[123]
Osteomyelitis	1	30	2	–	Early use of HBOT for a compromised host who develops recurrent osteomyelitis	[124]
Osteomyelitis	12	based on clinical outcome	2.5	90	Adjunctive therapy for patients who develop sternal infection and osteomyelitis after cardiothoracic surgery	[125]

ATA: atmospheres absolute, DFIs: Diabetic foot infections, HBOT: Hyperbaric oxygen therapy, NSTI: Necrotizing soft tissue infections, SSIs: Surgical site infections.

prospective study containing 125 burn patients, reported that HBOT has no significant effect on the rate of mortality, number of surgery, and length of hospitalization for the improvement of burn patients [39]. Mean healing times have been reported to be shorter in patients exposed to burn HBOT (mean: 19.7 days versus 43.8 days) [35]. The use of HBOT in conjunction with comprehensive burn management led to the significant control of sepsis in burn patients [40]. Shorter mean healing time and smaller fluid requirements have been reported in patients given HBOT [41]. Prospective studies with a larger number of patients are needed to confirm the role of HBOT in the treatment of extensive thermal burns.

### 3.5. Osteomyelitis

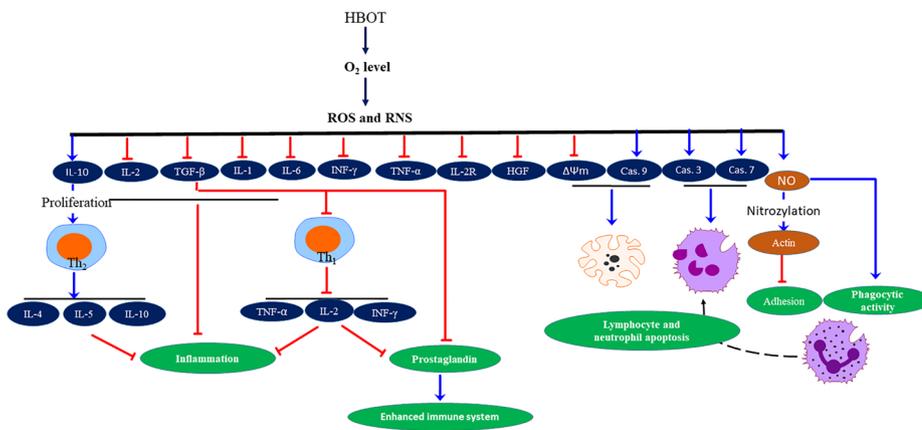
Osteomyelitis is defined as infections of the bone or marrow by bacterial pathogens. The treatment of osteomyelitis is difficult due to the relative paucity of blood vessels in bone and the fact that antibiotics often do not sufficiently penetrate bone [42]. The chronic osteomyelitis is a form characterized by the persistence of pathogens, mild inflammatory response, and the incidence of necrosis and fistulous tracts in bone tissue. Refractory osteomyelitis is a chronic bone infection that persists or reappears after applicable mediations have been completed. It is also referred to as refractory osteomyelitis when an acute form cannot be treated by confirmed management strategies, including antimicrobial and surgical intervention [43]. Remarkably, increased O<sub>2</sub> levels in the osteomyelitis lesion has been shown after inhalation of 100% O<sub>2</sub> during HBOT [44]. The refractory form of osteomyelitis has low frequency, thus it is more difficult to design randomized controlled trials in order to study the effects of HBOT on this infection [45]. A number of case series and cohort studies suggest that HBOT improves clinical outcomes of osteomyelitis [46–48]. HBOT might increase the effectiveness of refractory form of osteomyelitis treatment by several mechanisms, such as increased neutrophil activity, inhibition of

bacterial pathogens, enhanced antibiotic effects, decreased inflammation and enhanced healing mechanism. Inhibition of infection has been shown in 60–85% of patients with chronic, refractory, osteomyelitis after use of HBOT as adjunctive treatment [49].

### 3.6. HBOT for fungal infections

Recent estimates suggest that more than 3 million people have chronic or invasive fungal infections, causing more than 600,000 deaths every year [50]. Several factors contribute to poor outcomes in the treatment with antifungal drugs, such as modifications in the essential immune status of the patients, underlying primary disorders, time used for infection identification, heterogeneity in virulence characteristics of pathogens, and the condition of the infection site environment [51]. Attractive features of HBOT for severe fungal infections include its common clinical application for different conditions, guaranteed safety, and its noninvasive procedure [50]. Few *in vitro* and *in vivo* studies have demonstrated that HBOT is effective as an antifungal approach against *Aspergillosis* and *Zygomycosis* [52,53]. The reducing effect on biofilm through HBOT has been reported in *Aspergillus fumigatus* colonies *in vitro* through fungistatic mechanisms. Here, a lack of fungal superoxide dismutase (SOD) genes increased the effect of HBOT on fungal growth inhibition. However, no synergy was detected between HBOT and voriconazole or amphotericin B *in vitro* or *in vivo* with the dosing regimen tested [50]. Hypoxia conditions in the course of fungal infections and the obvious requirement for fungal adaptation to low levels of O<sub>2</sub> for host adaptation and virulence, show that further research on these mechanisms may prove to be clinically valuable. The effects of O<sub>2</sub> on fungal-host interactions might be complex and handling of O<sub>2</sub> concentrations and/or O<sub>2</sub> induced signaling pathways *in vivo* may have both helpful and harmful effects on the outcome of fungal infections [54]. Currently, it is unclear how increased levels of O<sub>2</sub> on the inhibition or promotion of fungal growth would affect the antifungal immune





**Fig. 3.** Enhancement of immune system antimicrobial effects by HBOT: The increased  $O_2$  levels during HBOT cause some cellular effects such as the suppression of proinflammatory mediators, transient decrease in the  $CD_4:CD_8$  T cell ratio, and prompting of lymphocyte and neutrophil apoptosis. In general, these effects can enhance the immune system's antimicrobial mechanisms and infection healing.

infection healing. Some monocyte/macrophage derived mediators may play a useful or detrimental role in the healing of infections. Impaired healing procedure, has been described by excessive inflammation associated with increased levels of  $TNF\alpha$  [80,81]. Some studies reported that healing improves by inhibition of excessive  $TNF\alpha$  expression [82,83]. However, such inhibition during HBOT could negatively influence host resistance to bacterial infection [84,85]. The precise consequences of such antagonistic crosstalk during HBOT, inflammation, infection healing and the host resistance to bacterial infection remains to be determined experimentally. Generally, the final effects of HBOT on different inflammation mediators, as well as resistance of the host to bacterial infections is not fully described and needs further laboratory and clinical observation. The anti-inflammatory property of HBOT may be due to the downregulation of  $IFN\gamma$ , PGs,  $TNF\alpha$ , IL-1, and IL-6 production [85]. The clearance of neutrophils from infected tissue is critical for the resolution of inflammation which happens via apoptosis [8]. The  $O_2$  level of the environment is a critical factor for the antibacterial activity of neutrophils. The bactericidal mechanism promotes potential respiratory bursts achieved via the production of superoxide radicals which needs large amounts of  $O_2$  [86]. There is a significant increase in  $O_2$  needed and consumption quantity during respiratory burst in infectious tissues. The induction of ROS formation and thus the antibacterial effect is depended on the local  $O_2$  partial pressure. This procedure, which is certainly the most essential defense mechanism against invading pathogens, is not effective under hypoxic circumstances. In addition, studies have reported that the pathogen burden in infectious tissues reduces consistently as  $O_2$  pressure is elevated. A single 90 min pre-treatment with HBOT induces the respiratory burst activity of neutrophil-like cells and increases phagocytosis of *Staphylococcus aureus* [8]. HBOT has a pro-apoptotic effect on neutrophils due to the induction of caspase 3/7 activity and morphological changes related to apoptosis. Both hyperoxia and pressure have been reported to contribute to the HBOT-induced promotion of antimicrobial activity and apoptosis of neutrophils by a non-consistent pattern [8]. Increased  $O_2$  after HBOT evidently increases bacterial killing capability of neutrophils. HBOT inhibits the adhesion of neutrophil. The adhesion of neutrophil is mediated by beta-integrin interaction with intercellular adhesion molecules (ICAM) on the endothelial surface. HBOT suppresses neutrophil beta-2 integrin (Mac-1 (CD11b/CD18)) activity by a nitric oxide (NO) mediated process and neutrophil counter ligand ICAM-1 on vascular endothelium [87,88]. This may be helpful in permitting neutrophil migration to the site of infections [49]. Inhibition of neutrophil beta-2 integrin is mediated via nitrosylation of actin, which is finally associated with HBOT induced increase in NO formation [8,89]. Phagocytosis of pathogens by neutrophils need a precise rearrangement of the actin cytoskeleton. The nitrosylation of actin was shown to stimulate the polymerization of actin. Therefore, it is believed that this could be the reason for the promotion of phagocytic activity of neutrophil subsequent HBOT pre-treatment [8]. HBOT prompts

apoptosis in the human Jurkat T-cell line by a mitochondrial pathway. Induction accelerated lymphocyte cell death has been reported after HBOT exposure via mitochondrial pathways. The inhibition of caspase-9, but not caspase-8, has been proven to block apoptosis induction by HBOT. These results show the immunomodulatory effect of HBOT [76].

#### 4.3. Synergistic effect with certain antimicrobial agents

In the clinical setting, HBOT is commonly administered in combination with antibiotic therapy in the treatment of an infection. Therefore, hyperoxia induction during HBOT may affect the activity of antibiotics [90]. It has been revealed that some bactericidal agents such as  $\beta$ -lactams, quinolones and aminoglycosides partly depend on bacterial aerobic metabolism in addition to their target-specific effects. Therefore, the efficiency of these drugs is influenced by the presence of  $O_2$  and the metabolic character of the pathogens [15]. The potential *in vivo*  $O_2$  concentration in the infectious tissues and its effect on antibiotic sensitivity of the pathogens are the key factors when setting susceptibility cutoff points for assessing the therapeutic property of an antimicrobial agent. It has been reported that low levels of  $O_2$  increase the resistance of *Pseudomonas aeruginosa* strains to piperacillin/tazobactam and *Klebsiella pneumoniae* strains to azithromycin. By contrast, some bacteria become more susceptible to tetracycline agents in the presence of low levels of  $O_2$  [91]. The aim of HBOT, as an alternative treatment, is to induce the aerobic metabolism of bacteria and to reoxygenate the  $O_2$ -depleted infectious tissues and therefore increase the microbial susceptibility to antibiotics [15]. Bacteria exposed to HBOT and simultaneously treated with antimicrobial agents exhibited significant changes in the cytoplasmic structure morphology; such as deformation and disorganization [92]. HBOT promotes aerobic metabolism leading to enhanced induction of ROS production in bacteria [15,93]. The administration of adjunctive HBOT twice a day with an 8 h' interval (280 kPa (2.8 bar) for 114 min) in combination with subcutaneous tobramycin (20 mg/kg/day) has shown a decrease in the bacterial load in *Staphylococcus aureus* infective endocarditis. Results have also shown decreased inflammatory reactions in rat models that indicate the potential effect of HBOT as an adjunctive therapy of *S. aureus* infective endocarditis [15]. HBOT (under the pressure of 3 ATA at 37 °C for 5 h) increased the effects of imipenem on *P. aeruginosa* infections of macrophages [92]. The combination of HBOT and cefazolin have shown to be more effective than cefazolin alone in the treatment of osteomyelitis caused by *S. aureus* in animal models [94]. HBOT, by re-oxygenation of biofilm, can considerably increase the bactericidal effect of ciprofloxacin on *P. aeruginosa* after 90 min of exposure. The combination of ciprofloxacin and HBOT therefore may potentially improve the eradication of *P. aeruginosa* biofilm in infectious tissue [95]. The enhanced bactericidal effect on *P. aeruginosa* biofilm of ciprofloxacin by HBOT is in part contributed by endogenous ROS formation as indicated by the higher susceptibility of a catalase-deficient mutant [96]. Significant

effect increases of vancomycin, teicoplanin, and linezolid in combination with HBOT have been reported against methicillin-resistant *Staphylococcus aureus* (MRSA) in an animal model mediastinitis [90]. Metronidazole is an antimicrobial agent that has been used for many years in the treatment of anaerobic and polymicrobial infection such as diabetic foot infections (DFIs) and surgical site infections (SSIs) [97,98]. The reduced form of metronidazole is effective against bacteria in an anaerobic circumstance [97]. The effect of HBOT in combination with metronidazole should be studied in the future through *in vitro* and *in vivo* studies.

### 5. The bactericidal effect of hyperbaric oxygen on antibiotic resistant isolates

Antimicrobial drugs tend to lose their effect over time due to the development and spreading of antibiotic resistant bacterial pathogens [3,99]. HBOT may be suitable for the treatment and prevention of multi-drug resistant pathogens and could be considered in cases of antibiotic therapy failure [100]. The bactericidal effect of HBOT against some clinically important drug resistant bacteria were reported. The exposure to HBOT (for 90 min at 2ATM) remarkably decreased the growth of MRSA [101]. HBOT also improved the antibacterial effect of several antimicrobial agents against MRSA infections in the rate model [90]. An obvious effective treatment of OXA-48 type carbapenemase-producing *K. pneumonia* osteomyelitis has been reported using HBOT without any concomitant antibiotics [100].

### 6. The complication of HBOT

The risks of O<sub>2</sub> toxicity depends on the level and intracellular localization of induced ROS. Due to the fact that exposure to hyperoxia in clinical HBOT procedures is rather brief, studies show that antioxidant responses are sufficient so that biological stresses induced by high levels of ROS are reversible. Induced damage of DNA by ROS appears to play a significant role in the stimulation of mutations and cancer. Under HBOT, the dissolved O<sub>2</sub> in the blood and also the generation of ROS are significant elevated. The exposure to high levels of O<sub>2</sub> may induce destructive effects in humans and it has been hypothesized that the toxic effects of excessive exposure to O<sub>2</sub> are related to an induced generation of ROS. The stimulation of oxidative DNA base injury by HBOT is well known DNA strand damage and oxidative base damage can be detected in peripheral blood, immediately, after a single session of HBOT, which demonstrates an increase in antioxidant defenses. DNA damage is not initiated when HBOT begins but is increases slowly after increased exposure time. To describe the antioxidant defenses after HBOT, exposed blood from subjects before and after HBOT with ROS generating mutagens has been studied and confirmed the premise of protective effects caused by HBOT that are not limited to a particular type of DNA damage [102,103]. This increased protection lasts for several days and in a cellular effect. The biochemical basis of this effect still has to be explained thoroughly but what is known is that antioxidants that scavenge ROS distant from nuclear DNA seem to be involved. The transcriptional response patterns to certain ROS are influenced on a cellular level, and 'classical' antioxidant responses that are promoted by high levels of ROS can be suppressed when cells adapt to low levels of ROS [104]. Assessment of oxidative effects of long-term repetitive HBOT on different brain regions of rats have been assessed by levels of lipid peroxidation and protein oxidation. Activities of superoxide dismutase and glutathione peroxidase have been suggested as an indicator of a strong protective mechanism against the hyperoxic condition, which is an adaptive reply for effective repair mechanisms [103]. This promotes an adaptive mechanism which defends lymphocytes against oxidative DNA damage prompted by a recurrent HBOT or by exposure to H<sub>2</sub>O<sub>2</sub>. The role of inducible enzyme heme oxygenase-1 (HO-1) has been demonstrated in this adaptive protection [105]. Increased levels of free iron due to HO-1 induction can promote increased levels of cellular

ferritin [106]. HBOT- exposed lymphocytes indicate a small but reproducible increase in cellular ferritin, which might suggest that the underlying protective response is established based on the stimulation of ferritin, which may act as antioxidant by inhibiting the formation of the DNA-damaging hydroxyl radical via the Fenton pathway [105]. HBOT often include so-called air breaks, where a patient respire only air for 5 min intervals once or twice throughout the course of the treatment [107]. Perhaps due to the particular atmospheric circumstance to which the individual is exposed, there are concerns about the side effects of HBOT. HBOT is safe if it does not exceed 2 h and the pressure does not exceed 3 ATM [108,109]. Potential side effects during HBOT, experienced most often with therapy of 4 ATA, include; barotraumatic lesions, O<sub>2</sub> toxicity, confinement anxiety and visual effects [109,110]. The main side effects are characterized by the presence of equalization disorder in the middle ear, however, serious complications rarely occur [110]. Patients treated by HBOT require careful pre-examination and monitoring. Absolute contraindications to HBOT include untreated pneumothorax (risk of becoming a tension pneumothorax), restrictive airway disorders (air becomes trapped with decompression and can lead to alveolar rupture with gas expansion), and simultaneous chemotherapy (has associated morbidity) [109]. If safety guidelines are strictly followed, HBOT is an effective modality with an acceptable rate of side effects.

### 7. Conclusion

HBOT is a primary or alternative option for the treatment of infections. Regarding an increased frequency of antibiotic resistant pathogen, HBOT can be effective in the treatment of acute infections. HBOT promotes the healing of infections by direct bacteriostatic or bactericidal effects, enhancement of immune system antimicrobial effects, and additive or synergistic effects with certain antimicrobial agents. If safety guidelines are strictly followed, HBOT is an effective procedure with an acceptable rate of side effects.

### Conflict of interest

There is no conflict of interest.

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### References

- [1] L.J. Piddock, The crisis of no new antibiotics—what is the way forward? *Lancet Infect. Dis.* 12 (3) (2012) 249–253.
- [2] D.J. Payne, M.N. Gwynn, D.J. Holmes, D.L. Pompliano, Drugs for bad bugs: confronting the challenges of antibacterial discovery, *Nat. Rev. Drug Discov.* 6 (1) (2007) 29.
- [3] M.Y. Memar, R. Pormehrali, N. Alizadeh, R. Ghotaslou, H. Bannazadeh Baghi, Colistin, an option for treatment of multiple drug resistant *Pseudomonas aeruginosa*, *Physiol. Pharmacol.* 20 (2) (2016) 130–136.
- [4] M.Y. Memar, P. Raei, N. Alizadeh, M.A. Aghdam, H.S. Kafil, Carvacrol and thymol: strong antimicrobial agents against resistant isolates, *Rev. Med. Microbiol.* 28 (2) (2017) 63–68.
- [5] M. Dryden, J. Cooke, R. Salib, R. Holding, S.L. Pender, J. Brooks, Hot topics in reactive oxygen therapy: antimicrobial and immunological mechanisms, safety and clinical applications, *J. Glob. Antimicrob. Resist.* 8 (2017) 194–198.
- [6] M.Y. Memar, R. Ghotaslou, M. Samiei, K. Adibkia, Antimicrobial use of reactive oxygen therapy: current insights, *Infect. Drug Resist.* 11 (2018) 567.
- [7] J. Shah, Hyperbaric oxygen therapy, *J. Am. Col. Certif. Wound Spec.* 2 (1) (2010) 9–13.
- [8] A.J. Almzaiel, R. Billington, G. Smerdon, A.J. Moody, Effects of hyperbaric oxygen treatment on antimicrobial function and apoptosis of differentiated HL-60 (neutrophil-like) cells, *Life Sci.* 93 (2) (2013) 125–131.
- [9] K.W. Van Meter, The effect of hyperbaric oxygen on severe anemia, *Undersea Hyperb. Med.* 39 (5) (2012) 937.
- [10] K. Van Meter, A Systematic Review of the Application of Hyperbaric Oxygen in the Treatment of Severe Anemia: an Evidence-based Approach, (2005).

- [11] H. Bitterman, Bench-to-bedside review: oxygen as a drug, *Crit. Care* 13 (1) (2009) 205.
- [12] P.M. Tibbles, J.S. Edelsberg, Hyperbaric-oxygen therapy, *N. Engl. J. Med.* 334 (25) (1996) 1642–1648.
- [13] X. Bai, Z. Song, Y. Zhou, S. Pan, F. Wang, Z. Guo, M. Jiang, G. Wang, R. Kong, B. Sun, The apoptosis of peripheral blood lymphocytes promoted by hyperbaric oxygen treatment contributes to attenuate the severity of early stage acute pancreatitis in rats, *Apoptosis* 19 (1) (2014) 58–75.
- [14] C.A. Godman, K.P. Chheda, L.E. Hightower, G. Perdrizet, D.-G. Shin, C. Giardina, Hyperbaric oxygen induces a cytoprotective and angiogenic response in human microvascular endothelial cells, *Cell Stress Chaperones* 15 (4) (2010) 431–442.
- [15] C. Lerche, L. Christophersen, M. Kolpen, P. Nielsen, H. Trøstrup, K. Thomsen, O. Hyldegaard, H. Bundgaard, P.Ø. Jensen, N. Høiby, Hyperbaric oxygen therapy augments tobramycin efficacy in experimental *Staphylococcus aureus* endocarditis, *Int. J. Antimicrob. Agents* 50 (3) (2017) 406–412.
- [16] C.-E. Chen, J.-Y. Ko, C.-Y. Fong, R.-J. Juhn, Treatment of diabetic foot infection with hyperbaric oxygen therapy, *Foot Ankle Surg.* 16 (2) (2010) 91–95.
- [17] V.V. Bumah, H.T. Whelan, D.S. Masson-Meyers, B. Quirk, E. Buchmann, C.S. Enwemeka, The bactericidal effect of 470-nm light and hyperbaric oxygen on methicillin-resistant *Staphylococcus aureus* (MRSA), *Lasers Med. Sci.* 30 (3) (2015) 1153–1159.
- [18] M.P. Fielden, E. Martinovic, A.L. Ells, Hyperbaric oxygen therapy in the treatment of orbital gas gangrene, *J. Am. Assoc. Pediatr. Ophthalmol. Strabismus* 6 (4) (2002) 252–254.
- [19] M.T. Akhi, R. Ghotaslou, M. Asgharzadeh, M. Varshochi, T. Pirzadeh, M.Y. Memar, A.Z. Bialvaei, H.S.Y. Sofla, N. Alizadeh, Bacterial etiology and antibiotic susceptibility pattern of diabetic foot infections in Tabriz, Iran, *GMS Hyg. Infect. Control* 10 (2015).
- [20] M.T. Akhi, R. Ghotaslou, M.Y. Memar, M. Asgharzadeh, M. Varshochi, T. Pirzadeh, N. Alizadeh, Frequency of MRSA in diabetic foot infections, *Int. J. Diabetes Dev. 37* (1) (2017) 58–62.
- [21] A. Abdulrazak, Z.I. Bitar, A.A. Al-Shamali, L.A. Mobasher, Bacteriological study of diabetic foot infections, *J. Diabetes Complicat.* 19 (3) (2005) 138–141.
- [22] W. Zamboni, H. Wong, L. Stephenson, M. Pfeifer, Evaluation of hyperbaric oxygen for diabetic wounds: a prospective study, *Undersea Hyperb. Med.* 24 (1997) 175–180.
- [23] A.P. Duzgun, H.Z. Satir, O. Ozozan, B. Saylam, B. Kulah, F. Coskun, Effect of hyperbaric oxygen therapy on healing of diabetic foot ulcers, *J. Foot Ankle Surg.* 47 (6) (2008) 515–519.
- [24] D. Paramythiotis, H. Koukoutsis, N. Harlaftis, Necrotizing soft tissue infections, *Surg. Pract.* 11 (1) (2007) 17–28.
- [25] Q.A. Hussein, D.A. Anaya, Necrotizing soft tissue infections, *Crit. Care Clin.* 29 (4) (2013) 795–806.
- [26] J.S. Ustin, M.A. Malangoni, Necrotizing soft-tissue infections, *Crit. Care Med.* 39 (9) (2011) 2156–2162.
- [27] C.R. Soh, R. Pietrobon, J.J. Freiburger, S.T. Chew, D. Rajgor, M. Gandhi, J. Shah, R.E. Moon, Hyperbaric oxygen therapy in necrotizing soft tissue infections: a study of patients in the United States Nationwide Inpatient Sample, *Intensive Care Med.* 38 (7) (2012) 1143–1151.
- [28] D. Wilkinson, D. Doolette, Hyperbaric oxygen treatment and survival from necrotizing soft tissue infection, *Arch. Surg.* 139 (12) (2004) 1339–1345.
- [29] M.T. Akhi, R. Ghotaslou, S. Beheshtirouy, M. Asgharzadeh, T. Pirzadeh, B. Asghari, N. Alizadeh, A.T. Ostadgavahi, V.S. Somesaraei, M.Y. Memar, Antibiotic susceptibility pattern of aerobic and anaerobic bacteria isolated from surgical site infection of hospitalized patients, *Jundishapur J. Microbiol.* 8 (7) (2015).
- [30] M.T. Akhi, R. Ghotaslou, N. Alizadeh, S. Beheshtirouy, M.Y. Memar, High frequency of MRSA in surgical site infections and elevated vancomycin MIC, *Wound Med.* 17 (2017) 7–10.
- [31] M.T. Akhi, R. Ghotaslou, N. Alizadeh, M. Yekani, S. Beheshtirouy, M. Asgharzadeh, T. Pirzadeh, M.Y. Memar, *nim* gene-independent metronidazole-resistant *Bacteroides fragilis* in surgical site infections, *GMS Hyg. Infect. Control* 12 (2017) Doc13.
- [32] M.E. Inanmaz, K.C. Kose, C. Isik, H. Atmaca, H. Basar, Can hyperbaric oxygen be used to prevent deep infections in neuro-muscular scoliosis surgery? *BMC Surg.* 14 (1) (2014) 85.
- [33] M. Qadan, O. Akça, S.S. Mahid, C.A. Hornung, H.C. Polk, Perioperative supplemental oxygen therapy and surgical site infection: a meta-analysis of randomized controlled trials, *Arch. Surg.* 144 (4) (2009) 359–366.
- [34] M. Misiuga, J. Glik, M. Kawecki, I. Dziurzyńska, M. Ples, W. Labuś, M. Nowak, The Effect of Hyperbaric Oxygen Therapy on Burn Wounds Covered With Skin Allografts, (2016).
- [35] I.-H. Chiang, S.-G. Chen, K.-L. Huang, Y.-C. Chou, N.-T. Dai, C.-K. Peng, Adjunctive hyperbaric oxygen therapy in severe burns: experience in Taiwan Formosa water Park dust explosion disaster, *Burns* 43 (4) (2017) 852–857.
- [36] J. Wasiak, M. Bennett, H.J. Cleland, Hyperbaric oxygen as adjuvant therapy in the management of burns: Can evidence guide clinical practice? *Burns* 32 (5) (2006) 650–652.
- [37] P. Cianci, J.J. Slade, R.M. Sato, J. Faulkner, Adjunctive hyperbaric oxygen therapy in the treatment of thermal burns, *Undersea Hyperb. Med.* 40 (1) (2013) 89–108 Inc.
- [38] H.N. Korn, E.S. Wheeler, T.A. Miller, Effect of hyperbaric oxygen on second-degree burn wound healing, *Arch Surg* 112 (6) (1977) 732–737.
- [39] A.L. Brannen, J. Still, M. Haynes, H. Orlet, F. Rosenblum, E. Law, W.O. Thompson, A randomized prospective trial of hyperbaric oxygen in a referral burn center population, *Am. Surg.* 63 (3) (1997) 205–208.
- [40] E. Villanueva, M.H. Bennett, J. Wasiak, J.P. Lehm, Hyperbaric oxygen therapy for thermal burns, *Cochrane Libr.* (2004), <https://doi.org/10.1002/14651858.CD004727.pub2>.
- [41] G. Hart, R. O'reilly, N. Broussard, R. Cave, D. Goodman, R. Yanda, Treatment of burns with hyperbaric oxygen, *Surgery, Gynecol. Obstet.* 139 (5) (1974) 693.
- [42] M. Hanley, J. Cooper, Hyperbaric, Chronic Refractory Osteomyelitis, (2017).
- [43] C.G. Kaide, S. Khandelwal, Hyperbaric oxygen: applications in infectious disease, *Emerg. Med. Clin. North Am.* 26 (2) (2008) 571–595.
- [44] E. Oguz, S. Ekinci, M. Eroglu, S. Bilgic, K. Koca, M. Durusu, U. Kaldirim, S. Sadir, Y. Yurttas, G. Cakmak, Evaluation and comparison of the effects of hyperbaric oxygen and ozonized oxygen as adjuvant treatments in an experimental osteomyelitis model, *J. Surg. Res.* 171 (1) (2011) e61–e68.
- [45] G. Lam, R. Fontaine, F.L. Ross, E.S. Chiu, Hyperbaric oxygen therapy: exploring the clinical evidence, *Adv. Skin Wound Care* 30 (4) (2017) 181–190.
- [46] S. Lentrodt, J. Lentrodt, N. Kübler, U. Mödder, Hyperbaric oxygen for adjuvant therapy for chronically recurrent mandibular osteomyelitis in childhood and adolescence, *J. Oral Maxillofac. Surg.* 65 (2) (2007) 186–191.
- [47] R. Ahmed, M.A. Severson III, V.C. Traynelis, Role of hyperbaric oxygen therapy in the treatment of bacterial spinal osteomyelitis, *J. Neurosurg. Spine* 10 (1) (2009) 16–20.
- [48] J. Banky, L. Ostergaard, D. Spelman, Chronic relapsing *Salmonella* osteomyelitis in an immunocompetent patient: case report and literature review, *J. Infect.* 44 (1) (2002) 44–47.
- [49] H.W. Hopf, J. Holm, Hyperoxia and infection, *Best Pract. Res. Clin. Anaesthesiol.* 22 (3) (2008) 553–569.
- [50] S. Dhingra, J.C. Buckley, R.A. Cramer, Hyperbaric oxygen reduces *Aspergillus fumigatus* proliferation in vitro and influences in vivo disease outcomes, *Antimicrob. Agents Chemother.* 62 (3) (2018) e01953–17.
- [51] C. Lass-Flörl, M. Cuenca-Estrella, Changes in the epidemiological landscape of invasive mould infections and disease, *J. Antimicrob. Chemother.* 72 (Suppl\_1) (2017) i5–i11.
- [52] B. John, G. Chamilos, D. Kontoyiannis, Hyperbaric Oxygen as an Adjunctive Treatment for Zygomycosis, Elsevier, 2005.
- [53] E. Segal, M. Menhusen, S. Simmons, Hyperbaric oxygen in the treatment of invasive fungal infections: a single-center experience, *IMAJ-Ramat Gan* 9 (5) (2007) 355.
- [54] N. Grahl, K.M. Shepardson, D. Chung, R.A. Cramer, Hypoxia and fungal pathogenesis: to air or not to air? *Eukaryot. Cell* (2012) EC. 00031–12.
- [55] C.G. Kaide, S. Khandelwal, Hyperbaric oxygen: applications in infectious disease, *Emerg. Med. Clin. North Am.* 26 (2) (2008) 571–595.
- [56] F. Vatansaver, W.C. de Melo, P. Avci, D. Vecchio, M. Sadasivam, A. Gupta, R. Chandran, M. Karimi, N.A. Parizotto, R. Yin, Antimicrobial strategies centered around reactive oxygen species–bactericidal antibiotics, photodynamic therapy, and beyond, *FEMS Microbiol. Rev.* 37 (6) (2013) 955–989.
- [57] M. Dryden, Reactive oxygen therapy: a novel therapy in soft tissue infection, *Curr. Opin. Infect. Dis.* 30 (2) (2017) 143–149.
- [58] C. Dunnill, T. Patton, J. Brennan, J. Barrett, M. Dryden, J. Cooke, D. Leaper, N.T. Georgopoulos, Reactive oxygen species (ROS) and wound healing: the functional role of ROS and emerging ROS-modulating technologies for augmentation of the healing process, *Int. Wound J.* 14 (1) (2017) 89–96.
- [59] H. Sies, Strategies of antioxidant defense, *FEBS J.* 215 (2) (1993) 213–219.
- [60] D.J. Dwyer, M.A. Kohanski, J.J. Collins, Role of reactive oxygen species in antibiotic action and resistance, *Curr. Opin. Microbiol.* 12 (5) (2009) 482–489.
- [61] M. Çimşit, G. Uzun, Ş. Yıldız, Hyperbaric oxygen therapy as an anti-infective agent, *Expert Rev. Anti. Ther.* 7 (8) (2009) 1015–1026.
- [62] S.G. Joshi, M. Cooper, A. Yost, M. Paff, U.K. Ercan, G. Fridman, G. Friedman, A. Fridman, A.D. Brooks, Nonthermal dielectric-barrier discharge plasma-induced inactivation involves oxidative DNA damage and membrane lipid peroxidation in *Escherichia coli*, *Antimicrob. Agents Chemother.* 55 (3) (2011) 1053–1062.
- [63] E. Cabiscol Català, J. Tamarit Sumalla, J. Ros Salvador, Oxidative stress in bacteria and protein damage by reactive oxygen species, *Int. Microbiol.* 3 (1) (2000) 3–8 2000.
- [64] J. Cadet, T. Douki, D. Gasparutto, J.-L. Ravanat, Oxidative damage to DNA: formation, measurement and biochemical features, *Mutat. Res. Mol. Mech. Mutagen.* 531 (1) (2003) 5–23.
- [65] K.B. Beckman, B.N. Ames, Oxidative decay of DNA, *J. Biol. Chem.* 272 (32) (1997) 19633–19636.
- [66] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, *Oxid. Med. Cell. Longev.* 2014 (2014).
- [67] F.C. Fang, Antimicrobial reactive oxygen and nitrogen species: concepts and controversies, *Nat. Rev. Microbiol.* 2 (10) (2004) 820.
- [68] S.V. Avery, Molecular targets of oxidative stress, *Biochem. J.* 434 (2) (2011) 201–210.
- [69] E. Birben, U.M. Sahiner, C. Sackesen, S. Erzurum, O. Kalayci, Oxidative stress and antioxidant defense, *World Allergy Organ. J.* 5 (1) (2012) 9.
- [70] J. Tamarit, E. Cabiscol, J. Ros, Identification of the major oxidatively damaged proteins in *Escherichia coli* cells exposed to oxidative stress, *J. Biol. Chem.* 273 (5) (1998) 3027–3032.
- [71] E. Granowitz, E. Skulsky, R. Benson, J. Wright, Exposure to increased pressure or hyperbaric oxygen suppresses interferon-(gamma) secretion in whole blood cultures of healthy humans, *Undersea Hyperb. Med.* 29 (3) (2002) 216.
- [72] G. Weisz, A. Lavy, Y. Adir, Y. Melamed, D. Rubin, S. Eidelman, S. Pollack, Modification of in vivo and in vitro TNF- $\alpha$ , IL-1, and IL-6 secretion by circulating monocytes during hyperbaric oxygen treatment in patients with perianal Crohn's disease, *J. Clin. Immunol.* 17 (2) (1997) 154–159.
- [73] N. Bitterman, H. Bitterman, A. Kinary, Y. Melamed, N. Lahat, Effect of a single

- exposure to hyperbaric oxygen on blood mononuclear cells in human subjects, *Undersea Hyperb. Med.* 20 (3) (1993) 197–204 Inc.
- [74] N. Xu, Z. Li, X. Luo, Effects of hyperbaric oxygen therapy on the changes in serum sIL-2R and Fn in severe burn patients, *Chin. J. Plast. Surg. Burns* 15 (3) (1999) 220–223.
- [75] L. Spiegelberg, S.M. Swagemakers, W.F. van IJcken, E. Oole, E.B. Wolvius, J. Essers, J.A. Braks, Gene expression analysis reveals inhibition of radiation-induced TGF $\beta$ -signaling by hyperbaric oxygen therapy in mouse salivary glands, *Mol. Med.* 20 (1) (2014) 257.
- [76] S.U. Weber, A. Koch, J. Kankleit, J.-C. Schewe, U. Siekmann, F. Stüber, A. Hoeft, S. Schröder, Hyperbaric oxygen induces apoptosis via a mitochondrial mechanism, *Apoptosis* 14 (1) (2009) 97–107.
- [77] J.A. Buras, D. Holt, D. Orlow, B. Belikoff, S. Pavlides, W.R. Reenstra, Hyperbaric oxygen protects from sepsis mortality via an interleukin-10-dependent mechanism, *Crit. Care Med.* 34 (10) (2006) 2624–2629.
- [78] B. Rinaldi, S. Cuzzocrea, M. Donniacuo, A. Capuano, D. Di Palma, F. Imperatore, E. Mazzon, R. Di Paola, F. Sodano, F. Rossi, Hyperbaric oxygen therapy reduces the toll-like receptor signaling pathway in multiple organ failures, *Intensive Care Med.* 37 (7) (2011) 1110–1119.
- [79] M.D. Valls, B.N. Cronstein, M.C. Montesinos, Adenosine receptor agonists for promotion of dermal wound healing, *Biochem. Pharmacol.* 77 (7) (2009) 1117–1124.
- [80] S. Berksoy Hayta, K. Durmuş, E.E. Altuntaş, E. Yildiz, M. Hisarciklo, M. Akyol, The reduction in inflammation and impairment in wound healing by using strontium chloride hexahydrate, *Cutan. Ocul. Toxicol.* 37 (1) (2018) 24–28.
- [81] K. Rapala, The effect of tumor necrosis factor-alpha on wound healing. An experimental study, *Annales chirurgiae et gynaecologiae, Supplementum* (1996) 1–53.
- [82] Z. Zhang, G. Cao, L. Sha, D. Wang, M. Liu, The efficacy of sodium aescinate on cutaneous wound healing in diabetic rats, *Inflammation* 38 (5) (2015) 1942–1948.
- [83] S.G. Gürgen, O. Sayin, F. Çetin, A.T. Yücel, Transcutaneous electrical nerve stimulation (TENS) accelerates cutaneous wound healing and inhibits pro-inflammatory cytokines, *Inflammation* 37 (3) (2014) 775–784.
- [84] M. Rayamajhi, J. Humann, S. Kearney, K.K. Hill, L.L. Lenz, Antagonistic crosstalk between type I and II interferons and increased host susceptibility to bacterial infections, *Virulence* 1 (5) (2010) 418–422.
- [85] N.S. Al-Waili, G.J. Butler, Effects of hyperbaric oxygen on inflammatory response to wound and trauma: possible mechanism of action, *Sci. World J.* 6 (2006) 425–441.
- [86] C.C. Winterbourn, A.J. Kettle, M.B. Hampton, Reactive oxygen species and neutrophil function, *Annu. Rev. Biochem.* 85 (2016) 765–792.
- [87] J. Kalns, J. Lane, A. Delgado, J. Scruggs, E. Ayala, E. Gutierrez, D. Warren, D. Niemeyer, E.G. Wolf, R.A. Bowden, Hyperbaric oxygen exposure temporarily reduces Mac-1 mediated functions of human neutrophils, *Immunol. Lett.* 83 (2) (2002) 125–131.
- [88] J.A. Buras, G.L. Stahl, K.K. Svoboda, W.R. Reenstra, Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS, *Am. J. Physiol.-Cell Physiol.* 278 (2) (2000) C292–C302.
- [89] S.R. Thom, M. Yang, V.M. Bhopale, S. Huang, T.N. Milovanova, Microparticles initiate decompression-induced neutrophil activation and subsequent vascular injuries, *J. Appl. Physiol.* 110 (2) (2010) 340–351.
- [90] V. Turhan, S. Sacar, G. Uzun, M. Sacar, S. Yildiz, N. Ceran, R. Gorur, O. Oncul, Hyperbaric oxygen as adjunctive therapy in experimental mediastinitis, *J. Surg. Res.* 155 (1) (2009) 111–115.
- [91] S. Gupta, N. Laskar, D.E. Kadouri, Evaluating the effect of oxygen concentrations on antibiotic sensitivity, growth, and biofilm formation of human pathogens, *Microbiol. Insights* 9 (2016) 37.
- [92] F.L. Lima, P.P. Joazeiro, M. Lancellotti, L.M. De Hollanda, B. de Araújo Lima, E. Linares, O. Augusto, M. Brocchi, S. Giorgio, Effects of hyperbaric oxygen on *Pseudomonas aeruginosa* susceptibility to imipenem and macrophages, *Future Microbiol.* 10 (2) (2015) 179–189.
- [93] M.A. Kohanski, D.J. Dwyer, B. Hayete, C.A. Lawrence, J.J. Collins, A common mechanism of cellular death induced by bactericidal antibiotics, *Cell* 130 (5) (2007) 797–810.
- [94] V. Mendel, B. Reichert, H. Simanowski, H.-C. Scholz, Therapy with hyperbaric oxygen and cefazolin for experimental osteomyelitis due to *Staphylococcus aureus* in rats, *Undersea Hyperb. Med.* 26 (3) (1999) 169.
- [95] M. Kolpen, N. Mousavi, T. Sams, T. Bjarnsholt, O. Ciofu, C. Moser, M. Kühl, N. Høiby, P.Ø. Jensen, Reinforcement of the bactericidal effect of ciprofloxacin on *Pseudomonas aeruginosa* biofilm by hyperbaric oxygen treatment, *Int. J. Antimicrob. Agents* 47 (2) (2016) 163–167.
- [96] M. Kolpen, C.J. Lerche, K.N. Kragh, T. Sams, K. Koren, A.S. Jensen, L. Line, T. Bjarnsholt, O. Ciofu, C. Moser, M. Kühl, N. Høiby, P.Ø. Jensen, Hyperbaric oxygen sensitizes anoxic *Pseudomonas aeruginosa* biofilm to ciprofloxacin, *Antimicrob. Agents Chemother.* 61 (11) (2017) e01024–17.
- [97] R. Ghotaslou, H.B. Baghi, N. Alizadeh, M. Yekani, S. Arbabi, M.Y. Memar, Mechanisms of *Bacteroides fragilis* resistance to metronidazole, *Infection, Genet. Evol.* 64 (2018) 156–163.
- [98] R. Ghotaslou, M. Yekani, M.Y. Memar, The role of efflux pumps in *Bacteroides fragilis* resistance to antibiotics, *Microbiol. Res.* 210 (2018) 1–5.
- [99] G.M. Rossolini, F. Arena, P. Pecile, S. Pollini, Update on the antibiotic resistance crisis, *Curr. Opin. Pharmacol.* 18 (2014) 56–60.
- [100] E. Goerger, E. Honnorat, H. Savini, M. Coulange, E. Bergmann, F. Simon, P. Seng, A. Stein, Anti-infective therapy without antimicrobials: apparent successful treatment of multidrug resistant osteomyelitis with hyperbaric oxygen therapy, *IDCases* 6 (2016) 60.
- [101] I. Tsuneyoshi, W.A. Boyle Iii, Y. Kanmura, T. Fujimoto, Hyperbaric hyperoxia suppresses growth of *Staphylococcus aureus*, including methicillin-resistant strains, *J. Anesth.* 15 (1) (2001) 29–32.
- [102] A. Rothfuß, C. Drennig, G. Speit, Adaptive protection against the induction of oxidative DNA damage after hyperbaric oxygen treatment, *Carcinogenesis* 19 (11) (1998) 1913–1917.
- [103] K. Simsek, M. Ozler, A.O. Yildirim, S. Sadir, S. Demirbas, M. Oztosun, A. Korkmaz, H. Ay, S. Oter, S. Yildiz, Evaluation of the oxidative effect of long-term repetitive hyperbaric oxygen exposures on different brain regions of rats, *Sci. World J.* 2012 (2012).
- [104] M.D. Temple, G.G. Perrone, I.W. Dawes, Complex cellular responses to reactive oxygen species, *Trends Cell Biol.* 15 (6) (2005) 319–326.
- [105] A. Rothfuss, G. Speit, Investigations on the mechanism of hyperbaric oxygen (HBO)-induced adaptive protection against oxidative stress, *Mutat. Res. Mol. Mech. Mutagen.* 508 (1) (2002) 157–165.
- [106] R. Meneghini, Iron homeostasis, oxidative stress, and DNA damage, *Free Radic. Biol. Med.* 23 (5) (1997) 783–792.
- [107] S.R. Thom, Hyperbaric oxygen—its mechanisms and efficacy, *Plast. Reconstr. Surg.* 127 (Suppl. 1) (2011) 131S.
- [108] C.A. Heyneman, C. Lawless-Liday, Using hyperbaric oxygen to treat diabetic foot ulcers: safety and effectiveness, *Crit. Care Nurse* 22 (6) (2002) 52–60.
- [109] S. Hunter, D.K. Langemo, J. Anderson, D. Hanson, P. Thompson, Hyperbaric oxygen therapy for chronic wounds, *Adv. Skin Wound Care* 23 (3) (2010) 116–119.
- [110] C. Plafki, P. Peters, M. Almeling, W. Welslau, R. Busch, Complications and side effects of hyperbaric oxygen therapy, *Aviat. Space Environ. Med.* 71 (2) (2000) 119–124.
- [111] J. Bartek, A.S. Jakola, S. Skyrman, P. Förander, P. Alpkvist, G. Schechtman, M. Glimåker, A. Larsson, F. Lind, T. Mathiesen, Hyperbaric oxygen therapy in spontaneous brain abscess patients: a population-based comparative cohort study, *Acta Neurochir.* 158 (7) (2016) 1259–1267.
- [112] F. Barili, G. Polvani, V.K. Topkara, L. Dainese, F.H. Cheema, M. Roberto, M. Naliato, A. Parolari, F. Alamanni, P. Biglioli, Role of hyperbaric oxygen therapy in the treatment of postoperative organ/space sternal surgical site infections, *World J. Surg.* 31 (8) (2007) 1702–1706.
- [113] A. Larsson, J. Uusjärvi, F. Lind, B. Gustavsson, H. Saraste, Hyperbaric oxygen in the treatment of postoperative infections in paediatric patients with neuromuscular spine deformity, *Eur. Spine J.* 20 (12) (2011) 2217–2222.
- [114] M.E. George, N.M. Rueth, D.E. Skarda, J.G. Chipman, R.R. Quickel, G.J. Beilman, Hyperbaric oxygen does not improve outcome in patients with necrotizing soft tissue infection, *Surg. Infect.* 10 (1) (2009) 21–28.
- [115] P.R. Massey, J.V. Sakran, A.M. Mills, B. Sarani, D.D. Aufhauser Jr., C.A. Sims, J.L. Pascual, R.R. Kelz, D.N. Holena, Hyperbaric oxygen therapy in necrotizing soft tissue infections, *J. Surg. Res.* 177 (1) (2012) 146–151.
- [116] A. Shupak, S. Oren, I. Goldenberg, A. Barzilai, R. Moskuna, S. Bursztein, Necrotizing fasciitis: an indication for hyperbaric oxygenation therapy? *Surgery* 118 (5) (1995) 873–878.
- [117] M. Londahl, P. Katzman, A. Nilsson, C. Hammarlund, Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes, *Diabetes Care* 33 (5) (2010) 998–1003.
- [118] E. Faglia, F. Favales, A. Aldeghi, P. Calia, A. Quarantiello, G. Oriani, M. Michael, P. Campagnoli, A. Morabito, Adjunctive systemic hyperbaric oxygen therapy in treatment of severe prevalently ischemic diabetic foot ulcer: a randomized study, *Diabetes Care* 19 (12) (1996) 1338–1343.
- [119] L. Kessler, P. Bilbault, F. Ortéga, C. Grasso, R. Passemard, D. Stephan, M. Pinget, F. Schneider, Hyperbaric oxygenation accelerates the healing rate of nonischemic chronic diabetic foot ulcers: a prospective randomized study, *Diabetes Care* 26 (8) (2003) 2378–2382.
- [120] L. Ma, P. Li, Z. Shi, T. Hou, X. Chen, J. Du, A prospective, randomized, controlled study of hyperbaric oxygen therapy: effects on healing and oxidative stress of ulcer tissue in patients with a diabetic foot ulcer, *Ostomy. Manage.* 59 (3) (2013) 18–24.
- [121] M. Kalani, G. Jörneskog, N. Naderi, F. Lind, K. Brismar, Hyperbaric oxygen (HBO) therapy in treatment of diabetic foot ulcers: long-term follow-up, *J. Diabetes Complicat.* 16 (2) (2002) 153–158.
- [122] A. Abidia, G. Laden, G. Kuhan, B. Johnson, A. Wilkinson, P. Renwick, E. Masson, P. McCollum, The role of hyperbaric oxygen therapy in ischaemic diabetic lower extremity ulcers: a double-blind randomised-controlled trial, *Eur. J. Vasc. Endovasc. Surg.* 25 (6) (2003) 513–518.
- [123] C.-E. Chen, S.-T. Shih, T.-H. Fu, J.-W. Wang, C.-J. Wang, Hyperbaric oxygen therapy in the treatment of chronic refractory osteomyelitis: a preliminary report, *Chang Gung Med. J.* 26 (2) (2003) 114–121.
- [124] L.A. Delasotta, A. Hanflik, G. Bicking, W.J. Mannella, Hyperbaric oxygen for osteomyelitis in a compromised host, *Open Orthop. J.* 7 (2013) 114.
- [125] W.-K. Yu, Y.-W. Chen, H.-G. Shie, T.-C. Lien, H.-K. Kao, J.-H. Wang, Hyperbaric oxygen therapy as an adjunctive treatment for sternal infection and osteomyelitis after sternotomy and cardiothoracic surgery, *J. Cardiothorac. Surg.* 6 (1) (2011) 141.