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Infectious Diseases: Pathophysiology and Mechanisms of Hyperbaric Oxygen

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Mader JT, Adams KR, Sutton TE. Infectious diseases: pathophysiology and mechanisms of hyperbaric oxygen. J Hyperbaric Med 1987; 2(3):133–140.—Hyperbaric oxygen (HBO) therapy has proved adjunctive along with antibiotics and surgery for the treatment of necrotizing soft tissue infections, refractory osteomyelitis, and infected ischemic wounds. The pathophysiology and mechanisms of HBO therapy explain these beneficial effects. Hyperbaric oxygen has a direct bactericidal effect on anaerobic organisms through the production of toxic oxygen radicals. Hyperbaric oxygen increases the oxygen tension in infected tissues which provides oxygen to the polymorphonuclear leukocytes to kill aerobic organisms. Hyperbaric oxygen provides oxygen to the fibroblast to allow new collagen formation and, subsequently, angiogenesis which allows hypoxic infected wounds to heal. Finally, HBO potentiates certain antibiotics such as the aminoglycosides and the sulfonamides.

hyperbaric oxygen, osteomyelitis, Staphylococcus aureus, Pseudomonas aeruginosa, tobramycin, polymorphonuclear leukocytes

Hyperbaric oxygen (HBO) has been used to treat a variety of infectious diseases. The beneficial mechanisms of hyperbaric oxygen include improved oxygen-dependent killing by the polymorphonuclear leukocytes, direct effect of toxic oxygen radicals on microorganisms, improvement in wound healing, and potentiation of certain antibiotics.

Oxygen is necessary for oxygen-dependent killing of microorganisms by the polymorphonuclear leukocytes and macrophages. These cells are the body's first line of defense against bacterial infections. Oxygen-dependent killing is initiated by the respiratory burst of the phagocytes. Its function is to produce a group of highly reactive microbicidal agents from the reduction of oxygen (1). The cornerstone of this reaction is reduced oxygen or superoxide. It is not clear if superoxide by itself is bactericidal, or if its role is to generate more potent oxygen radicals such as hydrogen peroxide, hydroxyl radicals, or singlet oxygen as the principal agents of killing. Regardless of the priority of potency, studies have shown that superoxide, hydrogen peroxide, hydroxyl radical, and singlet oxygen are all too reactive to be tolerated well within the living system (2).

Hydroxyl radicals are a highly unstable oxidizing species that react almost instantly with most organic molecules with which they come in contact. Hydroxyl radicals are bactericidal and may be formed by a reaction between superoxide and hydrogen peroxide (3).

Singlet oxygen must be regarded as a distinct chemical species with different patterns of reactivity. Singlet oxygen is highly reactive and capable of inflicting lethal damage on biological systems. During the respiratory burst, many products are formed that can react together to form singlet oxygen (4).

Another important process of the respiratory burst is the hydrogen peroxide-myeloperoxide-halide system. The enzyme myeloperoxide, found in phagocytic granules, catalyzes the oxidation of halide ions by hydrogen peroxide (5). Chloride halide is the most likely substrate, as it is most abundant in the cell. It is postulated that bacterial death may be facilitated in part by loss of integrity of the halogenated cell wall. Moreover, the myeloperoxidase system is able to decarboxylate amino acids, converting them to reactive aldehydes, carbon dioxide, and ammonia. The amino acid constituents of the bacterial cell wall are degraded by this reaction with resultant disruption of the cell wall (6).

Hydrogen peroxide and superoxide, the major toxic compounds produced by intracellular killing, must be detoxified by the polymorphonuclear leukocytes. Both hydrogen peroxide and superoxide can kill the host defense cells and damage biologic membranes (7, 8). The polymorphonuclear leukocyte possesses at least two cytoplasmic systems for detoxifying hydrogen peroxide: catalase and a coupled enzymatic reaction involving reduced glutathione. Superoxide anions are also toxic and can accumulate in the cell cytoplasm and in the extracellular medium surrounding the polymorphonuclear leukocyte. Superoxide is degraded by the enzyme, superoxide dismutase (9). Thus, the polymorphonuclear leukocyte seems protected from the cytotoxic effect of superoxide. The mechanisms for detoxifying the hydroxyl radical and singlet oxygen are unclear. These are highly reactive, short-lived oxygen radicals. Detoxification of hydrogen peroxide and superoxide in the polymorphonuclear leukocyte cytoplasm reduces production of these toxic oxygen radicals.

Exposure of intact leukocytes to a nitrogen atmosphere significantly reduces their microbicidal activity (10). However, this anoxic environment is not completely inhibitory, which indicates that oxygen-independent microbicidal systems contribute to phagocytic killing. The pH of the phagocytic vacuole is acidic (3.5–4.0) and may be lethal to some organisms (11). Granule release of lysozyme and lactoferrin inhibits growth of some microorganisms (12). Granule-associated hydrolytic enzymes released into the phagocytic vacuole attack proteins, carbohydrates, and lipids of the cells. Other cationic proteins are also released that demonstrate various methods of disturbing microorganism integrity (13). The relative importance of these oxygen-independent systems on bacterial killing is not well documented and varies from organism

to organism (14). However, for most microorganisms these systems are not as efficient and function more slowly than the oxygen-dependent killing mechanisms.

Hyperbaric oxygen therapy increases the oxygen tension in normal and infected tissue (15–17). Under standard HBO treatment conditions, the oxygen tension in infected tissue probably ranges from 30 to 1200 mmHg (18). However, current data are limited, and information is sparse for some tissue types. Under hyperbaric oxygen conditions, the natural defense mechanisms against oxygen radicals are overwhelmed with an increased production of these oxygen radicals (19). Increased oxygen radicals are produced intracellularly and are also found in the extracellular fluid. Development of tolerance to hyperoxia seems to correlate with increased superoxide dismutase and glutathione enzymes (20, 21) that reduce the toxic oxygen radicals. Little or no superoxide dismutase is present in extracellular fluid (22).

Most organisms that are termed aerobic bacteria are in fact facultative anaerobes. These organisms can live under reduced oxygen tensions as well as under normal oxygen tensions. Strict aerobes, in contrast, require oxygen for survival and division. As is common in the literature, facultative anaerobic and strict aerobic organisms will be designated as aerobic organisms throughout this discussion.

Hyperbaric oxygen increases the oxygen tension, which leads to the increased production of superoxide, hydrogen peroxide, and other oxygen radicals. However, Gregory and Fridovich (23) have shown that increased oxygen tensions induce further production of superoxide dismutase by aerobic organisms. Thus, aerobic organisms are able to detoxify superoxide even under hyperoxic conditions. In vitro studies, using standard HBO conditions, have shown no direct bactericidal effect on aerobic organisms with increased oxygen tension (24).

In addition to superoxide formation, HBO is known to modify tissue oxygen tensions. In infected areas, the tissue oxygen tension is markedly reduced (15, 25–27). However, standard HBO conditions increase the oxygen tensions to normal or above normal conditions in infected tissue (15). The oxygen-dependent intracellular killing mechanisms of the polymorphonuclear leukocyte require oxygen for adequate killing of many aerobic organisms. When the required oxygen is available in infected tissue through the use of standard HBO, polymorphonuclear leukocyte killing of aerobic organisms returns to normal or above normal.

Mandell (28) has shown that phagocytic killing of many aerobic organisms is diminished under hypoxic conditions. McRipley and Sbarra (29) demonstrated this for *Staphylococcus epidermidis* and *Pseudomonas aeruginosa;* Selvaraj and Sbarra (30) for *Escherichia coli;* and Hohn et al. (31) for *S. aureus.*

In our study (15) of experimental *S. aureus* osteomyelitis, HBO had no direct antibacterial effect. Bone oxygen tensions were measured both before and during hyperbaric oxygen in normal and osteomyelitic bone, using HBO

at normally safe treatment pressures (2 ATA, 100% oxygen). A mass spectrometer was used to obtain intramedullary bone oxygen tensions in normal and osteomyelitic bone, simultaneously. The oxygen tension was extremely low in osteomyelitic bone (23 mmHg). This effect was attributed to decreased perfusion and the presence of inflammation in the area of infection. In normal bone, the oxygen tension was significantly higher (45 mmHg). When animals were placed under HBO, the oxygen tension increased in both osteomyelitic (104 mmHg) and normal bone (322 mmHg).

Using the *S. aureus,* we found a spectrum of in vitro phagocytic killing under different oxygen tensions. The least amount of phagocytic killing occurred with 23 mmHg oxygen, the tension found in osteomyelitic bone under ambient conditions. Phagocytic killing increased when the oxygen tension was raised to 45 mmHg oxygen, the tension found in normal bone under ambient conditions. Further increase in phagocytic killing occurred when the oxygen tension was increased to 109 mmHg, the tension found in osteomyelitic bone under hyperbaric conditions. The greatest phagocytic killing of *S. aureus* occurred at 150 and 760 mmHg oxygen. If normal tissue oxygen tension (45 mmHg) is used as the condition under which "normal" phagocytic killing occurs, HBO therapy produces a tissue oxygen (109 mmHg) tension under which increased levels of phagocytic killing occurs. Unfortunately, most studies directed toward "normal" phagocytic killing have been performed under ambient oxygen conditions (150 mmHg) instead of tissue oxygen tensions (45 mmHg).

By increasing the intramedullary oxygen tension to levels where phagocytic killing proceeded more efficiently, HBO was effective in eradicating experimental staphylococcal osteomyelitis. Hyperbaric oxygen may have a similar indirect effect on other aerobic organisms by restoring to normal or above normal the intracellular killing mechanisms of the polymorphonuclear leukocyte, and possibly the monocyte-macrophage.

Increasing the oxygen tension has a direct lethal effect on strict anaerobic organisms and some microaerophilic aerobes. During HBO therapy, an increase in oxygen tension leads to the increased concentration of superoxide both intracellularly and extracellularly. Increased superoxide levels lead to increased production of hydrogen peroxide and other toxic oxygen radicals. Anaerobic organisms are extremely sensitive to these oxygen radicals because most lack the superoxide-degrading enzyme, superoxide dismutase, and the hydrogen peroxide-degrading enzyme, catalase (32). Thus, an increase in the oxygen tension with subsequent oxygen radical formation is lethal for most strict anaerobic organisms.

Hyperbaric oxygen has a direct bactericidal effect on most *Clostridia* species (33). In addition, HBO inhibits the further production of clostridial alpha toxin, a lecithinase, which can destroy cell membranes and increase capillary permeability (34). The effect of HBO has been less well documented with other strict anaerobic organisms. However, Hill (35) has shown a decrease in

number and size of experimental intraabdominal abscesses caused by *Bacteroides fragilis* and/or *Fusobacterium* sp. treated with HBO. Additionally, there have been several beneficial clinical reports on adjunctive HBO therapy for non-clostridial anaerobic infections (36–38).

Hyperbaric oxygen should augment the bactericidal action of the aminoglycoside class of antibiotics. These antibiotics include gentamicin, tobramycin, amikacin, and netilmicin. The aminoglycosides lack good antibacterial activity under low oxygen tensions. Adjunctive HBO increases tissue oxygen tensions, which should allow the aminoglycosides to kill more effectively. This hypothesis was studied in vitro and in vivo (39, 40) at the University of Texas Medical Branch at Galveston. Growth and killing studies were done aerobically and anaerobically with a *P. aeruginosa* clinical isolate at a concentration of 2×10^4 colony forming units (CFU)/ml, and a tobramycin level of 2 μg/ml. Aerobically, the tobramycin tubes showed a rapid decline in CFU by 6 h and complete killing by 24 h. The growth control tubes showed a 5 log increase in CFU by 24 h. Anaerobically, both the growth tubes and the tobramycin tubes showed growth nearly identical to the aerobic control tubes. Next, 1 h postinoculation, 100% oxygen was bubbled through the anaerobic tubes for 90 min. Nitrogen followed the oxygen for 20 min to restore the anaerobiasis. The tobramycin tubes showed a slow decrease in CFU by 6 h and a slow increase over the next 18 h, which was 2 logs lower than the control tubes. These in vitro experiments demonstrated that elevating oxygen tensions above hypoxic levels will partially restore the tobramycin killing of *P. aeruginosa*.

Next, the effect of tobramycin, hyperbaric oxygen, and hyperbaric oxygen plus tobramycin were studied in an osteomyelitis rabbit model (41). The animals were infected with *P. aeruginosa,* and the bone infection was allowed to progress for 2 wk before therapy was begun. Tibias were removed, ground, and cultured quantitatively at start of therapy and after 1, 2, and 4 wk of therapy. The results of this study are shown in Table 1. There was no decrease in CFU in the control or in the HBO treatment groups. The tobramycin group demonstrated greater killing at 4 wk than either the control or the HBO group ($P < 0.05$). The greatest improvement was found in the HBO and tobramycin combination therapy group. At all times, the combination therapy group yielded lower bacterial counts than did the control, HBO, or tobramycin groups ($P < 0.05$). Thus, the presence of adjunctive HBO seems to enhance the activity of tobramycin in the eradication of experimental *P. aeruginosa* osteomyelitis. Adjunctive HBO may also potentiate the bactericidal effect of vancomycin. Vancomycin, like the aminoglycosides, does not kill microorganisms well under low oxygen tensions. Finally, Keck et al. (42) have shown that HBO will potentiate the effect of certain sulfonamides.

Oxygen is also important in wound healing. When the environment of the fibroblast has an oxygen tension of less than 10 mmHg, the cell can neither synthesize collagen nor migrate. When the oxygen tension is increased the

TABLE 1: Experimental *P. aeruginosa* Osteomyelitis: Quantitative Tibial Counts

Groups	Week 1	Week 2	Week 4
Control	5.40 ± 0.22	5.59 ± 0.30	6.00 ± 0.19
Tobramycin	4.89 ± 0.34	4.98 ± 0.39	4.27 ± 0.31
Hyperbaric oxygen	5.74 ± 0.29	5.13 ± 0.21	5.81 ± 0.31
Combination therapy	3.92 ± 0.50	3.87 ± 0.32	3.38 ± 0.27

The effect of tobramycin, hyperbaric oxygen, or the combination of HBO and tobramycin on experimental *P. aeruginosa* osteomyelitis. The results of the quantitative *P. aeruginosa* tibial counts for each group are expressed as the mean and SEM log base 10. The mean *P. aeruginosa* tibial count at the beginning of therapy was 5.24 ± 0.19. Tobramycin was given at 5 mg/kg twice a day. Hyperbaric oxygen was given twice a day for 95 min at 2.5 ATA.

fibroblast can again carry out its functions (43, 44). The collagen produced forms a protective fibrous matrix, and new capillaries grow into this matrix. Since wound healing is a dynamic process, an adequate oxygen tension is mandatory for this process to continue. Hyperbaric oxygen therapy provides oxygen to promote collagen production, angiogenesis, and ultimately wound healing in the ischemic or infected wound. Hyperbaric oxygen is not necessary in a noncompromised wound.

In conclusion, HBO increases the oxygen tension in infected tissue, including bone. Hyperbaric oxygen has a direct lethal effect on strict anaerobic organisms through the production of toxic radicals. However, HBO has no direct effect on aerobic organisms. In fact, hyperoxic conditions induce aerobic organisms to produce increased concentrations of superoxide dismutase, an oxygen radical detoxifying enzyme. Hyperbaric oxygen increases the oxygen tension in infected tissue which provides the needed oxygen for the oxygen-dependent intracellular killing mechanisms of the polymorphonuclear leukocyte. Thus, HBO provides the necessary substrate (oxygen) for killing aerobic organisms by the polymorphonuclear leukocyte. Hyperbaric oxygen also augments the killing of the aminoglycoside tobramycin and certain sulfonamides. Finally, HBO provides adequate oxygen for fibroblast activity, leading to angiogenesis and wound healing in hypoxic tissues.

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