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BASIC MECHANISM OF HYPERBARIC OXYGEN IN INFECTIOUS DISEASE

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ABSTRACT

Hyperbaric oxygen therapy (HBOT) is the inhalation of 100 percent oxygen inside a hyperbaric chamber that is pressurized to greater than 1 atmosphere (atm). HBOT causes both mechanical and physiologic effects by inducing a state of increased pressure and hyperoxia. HBOT is typically administered at 1 to 3 atm. While the duration of an HBOT session is typically 90 to 120 minutes, the duration, frequency, and cumulative number of sessions have not been standardized. HBO has been use widely in treating gangrene diabetic, stroke, osteomyelitis and accelerating wound healing. The use of HBO in infectious disease is wide, so the mechanism of hyperbaric oxygen in infectious disease should be well-understand. This understanding could bring the proper and wise management of infectious disease and to prevent the side effect of each therapy.

Key words: *HBO, infectious disease, mechanism, proper and wise mechanism*

INTRODUCTION

This review would discuss the basic mechanism of action of hyperbaric oxygen in infectious disease. It will present the evidence for the bacteriostatic and bactericidal effect of hyperoxia and hyperbaric oxygen on microbial organisms in vitro and in in vivo model of infections. It will also examine the effect of oxygen on the activity of antimicrobial agent and on the function of immune defense mechanisms.

Regulation of Oxygen Delivery to Tissues

Tissue oxygen tensions are effected mainly by the concentration on inspired oxygen , cardiac output, local blood flow, cellular metabolism and substrate availability. (Kehrer JP *et al*, 1990; Sheffield PJ, 1988; Silver IA, 1984). Different partial pressures of oxygen (pO_2) are normally found in various body compartment. The pO_2s may the oven lower. In bacterial osteomyelitis, the pO_2 range from approximately 100 mm Hg within pulmonary alveoli to 15 mm Hg in the liver parenchymal cell. In traumatized or septic tissues, pO_2s may be even lower. In bacterial osteomyelitis, the pO_2s of bone is lowered by 50%; in experimental abscesses pO_2s may measure as low as 0 mm Hg (Hays RC, Mandell GL, 1974) within individual cells, pO_2s are heterogeneous and are much lower than extracellular pO_2s. For example, pO_2s in mitochondria are less than 1 mm Hg (Wilson DF, Erecinska M, 1984).

Normoxia (15%–21%) is defined in this review as the fractional inspired oxygen (FIO_2) concentration necessary to maintain aerobic metabolism and homeostasis in the body. Oxygen tensions outside this normal range will be devined as follows: Anaerob (less than 0.01% O_2), hypoxia (12% O_2 or less), hyperoxia (45%–100% O_2), and hyperbaric oxygen (any O_2 tension greater than I atmosphere absolute pressure or 760 mm Hg).

General Mechanism of Action of Oxygen in Infections

Hyperoxia and hyperbaric oxygen (HBO) increase oxygen tensions in tissue to levels which inhibit microbial growth by inhibiting various microbial metabolic reactions. Hyperoxia and HBO by themselves also exert direct bacteriostatic and bactericidal effects on selected microorganisms because of increased generation of reactive oxygen species or free radical (Jamieson D et al, 1986; Raffin TA et al, 1977). Free radicals are lethal for microorganisms that ether lack or possess limited antioxidant defenses. HBO is a unique antibacterial agent.

At doses used clinically, HBO is usually bacteriostatic. Not all doses of HBO have an antibacterial effect. The use of HBO at pressures of 1.5 ATA or less promotes the growth of aerobic bacteria in vitro (Olodart RM, 1966).

Hyperbaric oxygen also raises oxygen tensions in hypoxic tissue to levels necessary for the killing of bacteria by neutrophils (Mader JT et. Al, 1980). While phagocytosis remains unaffected by low oxygen tensions (Karnovsky ML, 1968) killing of microorganisms by the oxidative burst is dependent on oxygen tensions. (Babior BM, 1978; Beaman L *et al*, 1984; Hasset DJ, Cohen MS, 1989) polymorphonuclear leukocytes (PMNs) from patients with chronic granulomatous disease lack the enzyme NADPH-oxidase necessary for oxygen –dependent killing of such pathogenic bacteria as *Pseudomonas aeruginosa and Staphylococces aereus* (Mandell GL, Hook EW, 1969).

Hyperoxia and HBO also influence the activity of selected antimicrobial agens belonging to the following categories antimetabolites, protein synthesis inhibitors and reduction – oxidation cycling agent. Oxygen tensions also influence the pharmacokinetics of antimicrobial agent. For example, hypoxemia (PaO_2-32mm Hg) prolongs (2-fold) the serum half –life of aminoglycosides hypoxemia effects both the absorption from muscle as well as the climination of these antimicrobials (Miphij MJ *et al*, 1978).

Hyperbaric oxygen can also effect the outcome of infections indirectly by influencing tissue repair and regeneration responses in infected necrotic tissue.for example, hypoxia (12% O_2, 1 ATA) retards healing of skins wounds and thus probably favors bacterial growth (Knighton DR *et al*, 1986) hyperbaric oxygen (100% O_2, 2 ATA, 2t, twice daily)does not effect the healing of vascularized, full-thickness skin wound , but enhances wound closure in ischemic wound (Kivisaari J, Niinikoski J, 1975).

Significantly decreased in uninfected bone of rabbits after exposure to HBO (100% O_2, 2ATA) (Stelner B *et al*, 1984) the hemodynamic changes induced by hyperbaric oxygen may be the results of increased oxygen delivery of tissue. It is also possible that negative inotropic effect on myocardium play a role in these changes. As for as can be judged from work with a model of antibiotic – controled pepsis , the presence of sepsis per se does not cause may hemodynamic changes during exposure to HBO (Muhvich KH, 1986).

Susceptibility of anaerobic and aerobic bacteria to HBO

Pathogenic bacteria are classified in terms of the partial pressure of oxygen in which they grow. By definition , anaerobic bacteria can not survive in normal oxygen tensions because they lack antioxidant defenses. As such they are very susceptible HBO . for example, hyperbaric oxygen (3 ATA for 18 hours) is completely bactericidal for *Clostridium perfringens* in vitro (Hill GB, Osterhaut S, 1972) however, there are differences in susceptibility to oxygen among Clostridium spesies. Hyperbaric oxygen (100% O_2, 2 ATA) block the germination of *C. perfringens* spores in vitro, but is not bactericidal for the spores (Demello FJ *et al*, 1970) facultative anaerobic bacteria are able to grow in normoxia hyperoxia by increasing the synthesis of antioxidant enzymes (Gregory EM, Fridovich I, 1973).

The growth of some aerobic bacteria is enhanced by hyperoxia, but is inhibited by HBO. For example, oxygen tentions upo to 1 ATA enhance the growth of *Escherichia coli*, whereas oxygen tensions greater than 2 ATA inhibit growth in vitro. (Olodart RM, 1966) hyperoxia (100% O_2, 1 ATA) enhances the the growth of *P. aerogenesa* in vitro (Park MK et. Al, 1991); hyperoxia (0.2 ATA to 0.87 ATA) enhances the growth of *Corynebacterium diphtheriae* in vitro (Gottlieb SF *et al,* 1974).

Prolonged in vitro exposure to oxygen tensions greather than 1.5 ATA inhibith the growth of several aerobic and facultative anaerobic bacteria. Hyperbaric oxygen (greather than 1.5 ATA) is bacteriostatic for *E. coli* (Bochme DE *et al*, 1976; Brown OR, 1972) *P. aerugenesa*, (Bornside GH *et al*, 1975, *C. diphtheriae, Lactobacillus casei*, (Gottlieb SF, 1979) and Vibrio anguillarum (Keck PE *et al*, 1980) however, a 1 hour intermittent exposure to HBO (100% O_2, 2 ATA every 8 hours) has no effect on the growth of *Pherugenosa* or *S. aureus* (Brown GL *et al*, 1979) prolonged in vitro hyperbaric oxygen exposure (2.9 ATA O_2, 24 hours) is also bacteriostatic for the following enteric bacteria *Salmonella thyposa*, *S. schottmuelleri, S. paratyphi, shigella dysenteriae, S.flexneri,* and *Proteus vulgaris* (Bornside *et al*, 1975) the growth of *Strepstococcus (enterococcus) faeculis* is partially in habited by 2.9 ATA O_2 however, an alpha hemolytic strain of streptococcus is not inhibited by HBO (Gottlieb SF, 1979) possibly because of the presebse of a hyaluronic acid-containing capsule (Cleary PP, Larkin A, 1979).

Hyperbaric oxygen is bactericidal for aerobic and facultative anaerobic bacteria usually only at pressures and/ or durations which are greather than can be used clinically. For example, HBO is bactericidal for *P.aeroginosa , Proteus vulgaris , and S. typhosa.* at 3 ATA for 24 hours and for E. coli at 20 ATA when treated for 6 hours (Bornside *et al*, 1975).

Mechanisms of Bacteriostatic Effect of HBO

HBO inhibits the growth of aerobic facultative anaerobic bacteria by inducing a variety of metabolic effect involved with the syntesis of proteins. Nucleic acids and essential cofactors metabolic reactions: membrane transport function are also effected. These effects where achieved with the use of hyperbaric oxygen in viyo.

Inhibition of Amino Acid and Protein Biosyntesis

Exposure of E. coli to hyperbaric oxygen (100% O_2 at greater than 3 ATA) causes a rapid inhibition of growth and respiration (Brown OR, 1972) the inhibitory effect HBO are most likely caused by free radicals and other reactive oxygen based molecules, because hyperoxia (100% O_2, 1 ATA) inhibits growth of a superoxide dismutase-deficient double mutant of E.coli (sod A sod B) (Carlioz A, Touati D, 1986) free radicals probably

inactivate a bacterial enzyme (dihydroxyacid dehyoratase) involved in amino acid biosynthesis (Brown OR, 1975). Dihydroxyacid dehydratase catalizes the formation of alpha-ketoisovalerate, an intermediate in the formation of valine and leucine. Hyperbaric oxygen (100% O_2 4.2 ATA) deceases the specific activity of dihydroxyacid dehydratase by 78 % (Brown OR, 1975). The inhibition of amino acid biosynthesis by HBO eventually leads to increase level of tRNA, which is responsible for inducing stringency response.The stringency response is characterized by increased level of tetra- and penta- phosphorylated guanosine which inhibit bacterial carbohydrate, lipid and nucleotide synthesis and enhance proteolysis (Cashel M, 1975). The end result is cessation of bacterial growth.

The inhibition by HBO of protein synthesis in bacteria may also be caused by free radical- induced block in the transport of substrates use in RNA transcription. Hyperoxia or the superoxide anion free radical inhibit the transport of lactose, guanosine and methylglycopyranoside in to E.coli (Forman HJ *et al*, 1982). Hyperoxia also inhibit the transport of protons and the synthesis of ATP in bacterial membranes (Wilson DM *et al*, 1976). However it appears that the growth inhibition caused by HBO begins long before a drop in ATP level occurs (Mathis RR, 1976). The mechanism of decreased transport cused by HBO is thought to be the oxidation of sulfhydryl-containing protein involved in transport of metabolic substrates. Free radicals are able to inactivate other bacterial proteins with key enzymetic function by oxidizing sulfhydryl-containing animo acids such as metionine play a key role in defending against this type of oxidative damage to proteins (Brot N *et al*, 1981).

Decreased Levels of Key Cofactors of Metabolic Reactions

Hyperbaric oxygen also inhibits bacterial growth by decreasing the levels of thiamine and of both the reduced and oxidiced forms of nicotinamide adenine dinnucleootide (NAD, NADH) (Brown OR, 1983) thiamine pyrophosphate is an essentral coenzyme in carbohydrate metabolism and NADPH production; NADPH is a critical cofactor in a wide range of metabolic reactions. The mechanism of the decrease in NAD is in inhibition of the de nooa NAD syntesis pathway and possibly also and increase in catabolism of NAD (Gardner PR, 1990).

Decreased Synthesis in Increased Degradation of DNA and RNA

Hyperbaric oxygen can also inhibit bacterial growth by directly blocking RNA transcription and DNA syntesis, for example, HBO (4,2 ATA) inhibits RNA transcription and DNA systhensis in both stringent and relaxed strains of E. coli after a 30 minute exposure (Brown OR, 1983).

Electron microscopic studies show ultrastructural evidence of degradation of nucleic acids and ribosomal proteins in *P.aeruginosa*, after bacteriostasis induced by prolonged exposure to HBO (100% O_2 ,2.9 ATA) for 24 hours (Clark JM, 1971). *P. aerugioesa* undergo market changes in morphologic appearance when exposed to oxygen at pressures that do not induce bacteriostasis (100% O_2 ,2 ATA). These abnormal shape changes are reversible (Kenward MA *et al*, 1980).

Another important mechanism of oxygen-induced toxicity to bacteria is via injury to DNA. Production of to superoxide anion in vitro and in vivo has been linked to mutations in bacteria, HBO is mutagenic induced the reversion of a tryptophan auxotroph (E.coli WP 2 hcr) to prototrophy. Paraquat toxicity for E. coli is in large part due to superoxide radical production (Hassan and Fridovich, 1978). Paraquat is highly mutagenic for two strains of S. typhimurium (Moody and Hassan, 1982). Both base-pair substitution and frameshift mutations were noted in DNA from the *Salmonella* strains. cell containing high levels of SOD are more resistant to toxicity and mutagenecity than cell containing normal levels of this enzyme.

From a quantitative standpoint, an important cellular source of superoxide is the nonenzymatic oxidation of cytochrome intermediates of the electron transport chain in mitochondria.

Superoxide is also generated by the cytochrome-P-450 substrate-oxygen complexes in the endoplasmic reticulum. Another cellular organelle producing tonic oxygen spesies is the peroxisome. Here H_2O_2 production occurs by oxidation of substrates such as long chain fatty acids. In all these cellular organelles, the generation of tonic oxygen spesies is dependent on tissue oxygen tensions (Turrens JF *et al*, 1982) Xantine oxidase is a major source of O_2 in ischemic and hypoxic tissue that undergo re- oxygenetation by blood reflow (McCord JM,1985). In summary, the presence of an adequate amound of molecular oxygen is necessary for oxygen- dependent killing by PMNs and macrophages to occur. A variety of enzymatic and nonenzymatic celluler reactions also normally result in the production of O_2 and H_2O_2. The production of these molecules is enhanced by increasing tissue oxygen tensions. Free radicals are highly reactive and if not removed by scavengers, may cause extensive cellular injury.

Bacterial Defense Mechanism Against Free Radicals

For protection against the free radicals generated during normal aerobic metabolism, cells have developed antioxidant defence mechanism. Three main antioxidant enzymes are known. Superoxide dismutase (SOD) is an extremely efficient O_2 (GSH peroxidase) catalyzes the reduction of hydrogen peroxide to water and dioxygen, and is capable of converting tonic lipid peroxides into nontonic products.

Superoxide onion may undergo spontaneous dismutation to form hydrogen peroxygen. The rate of reaction is enhanced markedly by the presence of superoxide dismutase (SOD). Dismutation of two O_2 radicals results in the formation of one hydrogen peroxide molecule. Catalase subsequently converts hydrogen peroxide to water and oxygen. The role of catalase is probably more important during hyperoxic conditions than in normoxic conditions. In the presence of trace amount of transition metals ,

particularly iron, hydrogen peroxide may participate in the Fenton reaction. This reaction serves to produce the highly reactgive OH. Radical, removal of H_2O_2 by catalase is important in order to prevent lipid peroxidation of membranes by OH.

Free radicals may also be inactivated by reacting with low molecular weight substances located in the cellular membranes or in the cytosol. Tocopherol (Vitamin E) is in antioxidant located in membranes. Ascorbate, beta-carotene and sulfhydryl-containing compound such as cysteine, cysteamine and gluthatione are water soluble antioxidant compound. Inder normal metabolic conditions, these free radical cellular injury. However, if host devense mechanism atre overwhelmed, damage to eukaryotic cells as well as procarotic cells will occur. (Freeman BA, 1982).

It is clear that primary mechanism of toxicity of HBO for eukaryotic cells and for microorganism is through the generation of free radicals, and other tonic oxygen spesies. Mammalian cells have various antioxidant defense and utilize free radical reactions for bacterial killing. Augmentation of endogenous host antioxidant defenses may permit use of higher doses of HBO than are currently possible in the treatment of infectious disease states. One of the rationales for using hyperbaric in infections is the potential to exploit the enhanced of selected microorganism to tonic oxygen molecules.

Role of Superoxide and Hydrogen peroxide in Bacterial Killing by Hyperoxia and hyperbaric oxygen

The superoxide anion radical appears to be particularly important in bacterial killing (Gregory EM, 1974) several in vitro studies have shown that the absence of the enzyme responsible for the detoxification of O_2, namely superoxide dismutase (SOD), increases the susceptibility of many anaerobic and facultative anaerobic bacteria to oxygen (McCord JM, 1971) on the other hand , by raising bacterial levels of SOD , the susceptibility of the bacteria to oxygen can be diminished in vitro. For example, SOD levels in *B. fragilis* can be raised 5-fold by exposure to 2% O_2 (Privale CT, 1979). The increased SOD activity markedly reduces killing of these bacteria by HBO (Gregory EM, 1973) killing of *S. sanguis* can also be prevented by increasing SOD activity : dimenthylsulfoxide (apermeable OH. Scavenger) does not protect againt free radical toxicity (DiGuiseppi J, Fridrovich I, 1982) studies with SOD and catalase devicient mutants of E. coli confirm that SOD is more important than catalase in protecting against the growth inhibition caused by hyperoxia (Schellhorn HE, Hassan HM, 1988).

In some strains of bacteria such as L. plantarum high levels of Mn^{2+} appear to be an effective substitute for SOD in protecting against the toxic effect of O_2. other bacteria such as N. gonorrhoeace are particulary susceptible to a different toxic oxygen spesies, namely H_2O_2. in these bacteria resistance to oxygen induced killing is associated with high levels of catalase, the enzyme responsible for detoxification of H_2O_2 . Aditional antioxidant defence such as peroxidase and high levels glutathione also contribute to survival of these bacterial in aerobic conditions (Archibald FS, Duong MN, 1986).

Work done by Beaman et al,(1985) has shown that surface associated SOD and high levels of catalase in *Nocardia asteroides* act together to resist oxygen dependent microbicidal activity of human PMNs. Microoganism with adequate antioxidant defenses are resistant to toxic actions of O2 and may use the production of toxic oxygen spesies to injure host cells for example, virulent strains of listeria monocylogenis exhibit maximal production of H_2O_2 and O_2. Virulence is correlate with survival of *Listeria monocytogenesis* in macrophage monolayers. The exogeneous H_2O_2 damage macrophages. An avirulent strain of *L. monocytogenes* does not release H_2O_2 or O_2 in significant amounts (Godfrey RW, Wilder MS, 1985).

It is not clear if damage to bacterial cytoplasmic membrane caused by HBO is significant enough to be considered an important mechanism of HBO induced killing. In the case of E. coli, very few broken cells and no evidence of membrane lipid peroxidation are seen after the bacterial have been killed by HBO in vivo (Harley JB *et al*, 1981). However the presence of a capsule appears to protect bacteria against oxygen-induced damage, in the case of *Streptococcus pyogenes* the presence of a hyaluronic acid capsule increases resistance to the bacteriostatic effect of oxygen. Removal of the capsule from an encapsulated St*reptococcus* strain using hyaluronidase digestion increases susceptibility of this bacterium to the toxic effect of oxygen (Cleary PP, Larkin A, 1979).

Genetic Mechanism of Bacterial Resistance to Oxygen

Two regulatory genes responsible for the increased resistance of bacteria to hyperoxia have been indentified and are known as the soxR and oxyR regulons. Hyperoxia and superoxide induce the synthesis of 30 proteins; approximately 20 of these proteins are regulated by the soxR or the osyR regulons. (Christman MF *et al*, 1985; Greenberg JT *et al*, 1990; Storz G *et al*, 1990; Walkup LKB, Kogama T, 1989). Many of these bacterial proteins are enzymes involved in detoxification of free radicals and repair of free radicals damage; example are SOD , endonuclease IV, and glucose 6-phosphate dehydrogenase (Greenberg JT *et al*, 1990; Tsavena JR, Weiss B, 1990). Example of these proteins include the antioxidant enzymes hydroperoxidase 1 catalase. NAD(P)H- dependent alkyl hydroperoside reductase, and glutathione reductase, exposure to toxic oxygen species induces the synthesis of several other protective proteins whose specific identify remains to be characterized (Christman MF *et al*, 1985; Demple B, halbrook j, 1983; Storz G *et al*, 1990).

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