OKSIGEN HIPERBARIK (OHB) MENYEMBUHKAN SEL MELALUI SPESIES OKSIGEN REAKTIF (SOR)

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ABSTRACT

In degenerative diseases, hypoxia is the fundamental mechanism. Hypoxia stimulates reactive oxygen species (ROS) production. ROS interacts with protein, lipid, DNA, and other cell's components and cause damage. Hyperbaric oxygen (HBO) improves clinical outcomes but the exact mechanism is still debated. HBO reverses hypoxia state. HBO increases ROS production because of hyperoxia but it can heal the cell. HBO-induced ROS stimulates endogenous antioxidants, hypoxia-inducible factors, and heat shock proteins. ROS theoretically improves telomere length and telomerase activity. Superoxide is one of ROS which converted into hydrogen peroxide and it acts as signal transduction. ROS has positive outcomes in maintaining cell survival.

Keywords*: Hyperbaric Oxygen, Reactive Oxygen Species, Antioxidant, Hypoxia-Inducible Factor, Heat Shock Protein*

ABSTRAK

Hipoksia adalah mekanisme fundamental pada penyakit degeneratif. Hipoksia menstimulasi produksi spesies oksigen reaktif (SOR). SOR berinteraksi dengan protein, lipid, DNA, dan komponen sel lainnya dan mengakibatkan kerusakan. Oksigen hiperbarik (OHB) memperbaiki luaran klinis namun mekanismenya masih diperdebatkan. OHB mengembalikan status hipoksia. OHB meningkatkan produksi SOR sebab efek hiperoksia namun hal tersebut mampu menyembuhkan sel. SOR yang terinduksi OHB menstimulasi antioksidan endogen, *hypoxia-inducible factors*, dan *heat shock proteins*. SOR secara teoritis memperbaiki panjang telomere dan aktivitas telomerase. Superoksida adalah salah satu SOR yang dikonversi menjadi hidrogen peroksida dan bertindak sebagai transduksi sinyal. SOR mempunyai luaran positif dalam mempertahankan kehidupan sel.

Kata Kunci: *Oksigen Hiperbarik, Spesies Oksigen Reaktif, Antioksidan, Hypoxia-Inducible Factor, Heat Shock Protein*

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INTRODUCTION

Hyperbaric oxygen (HBO) has been widely used as therapy in many clinical conditions. HBO uses pure oxygen with pressure more than 1 atmosphere (above sea level pressure) in hyperbaric chamber. Undersea and Hyperbaric Medical Society (UHMS) has already recommended indications for HBO therapy (1).

There are 14 indications (1):

1. Air or gas embolism
2. Decompression sickness
3. Carbon monoxide poisoning
4. Clostridial myositis and myonecrosis (gas gangrene)
5. Crush injury, compartment syndrome, and other acute traumatic ischemia
6. Arterial insufficiency
7. Severe anemia
8. Intracranial abcess
9. Necrotizing soft tissue infection
10. Osteomyelitis (refractory)
11. Delayed radiation injury (soft tissue and bone necrosis)
12. Compromised grafts and flaps
13. Acute thermal burn injury
14. Idiopathic sudden sensorineural deafness

The other clinical indications are classified as off label that HBO shows beneficial effects but still no concensus. HBO has physiological effects such as hyperoxia or hyperoxygenation, pressure effect, reduces edema, modulates defense mechanism, modulates inflammation process and immunity. Hyperoxia has been postulated increase reactive oxygen species (ROS) because of a lot of amount oxygen. Eventhough it is still debated, HBO therapy improves clinical outcomes. Measurement of ROS in patients after HBO therapy shows ROS or ROS-related products increase (2). HBO is proposed having hormetic mechanism. Hormesis theory defines the substance or agent has dual effects. It can be positive or negative effects. Hormesis theory mentions high dose is toxic and low dose is therapeautic. HBO can be toxic that they have been known as Paul-Bert effect (central nervous system toxicity) and Lorraine-Smith effect (pulmonary toxicity). HBO in therapeautic dose range heals cells and tissues. We will discuss how HBO-induced ROS can heals cell.

Reactive Oxygen Species

Free radicals have been known as cause of many pathological condition and also aging since Denham Harman introduced free radical theory. Free radical is a molecule with unpaired electron. Free radical reacts with other molecules to stabilize itself. The reactions change the structure of original molecules, so they change the original function. Free radicals

are believed as the factor which has negative effects in health. Recently, we understand the sources of free radicals in normal (physiological) condition. It is still debated why free radicals are produced in normal condition. Some experts argue the role of free radicals in physiological function especially as the transduction signals (3).

Free radicals consist of 2 forms. They are reactive oxygen species (ROS) and reactive nitrogen species (RNS). They are classified according to their basic molecule. ROS consists of hydroxyl radical ($\cdot OH^-$), superoxide ($\cdot O_2^-$), hydrogen peroxide (H_2O_2). Some experts classify hydrogen peroxide as nonradical oxidant because of its low toxicity. RNS consist of nitric oxide ($\cdot NO$), nitrogen dioxide ($\cdot NO_2$), peroxynitrite ($\cdot ONOO^-$).

Mitochondria is the largest source of ROS. They utilize almost 95% oxygen to produce energy. ROS is formed because of electrons transfer process in respiratory chain. In 1972, Harman revised the free radical theory into mitochondria free radical theory or mitochondria theory. ROS was previously as a cause then change as a consequence. Mitochondria was proposed as the cause of diseases (4).

The generation of ROS (5):

(oxidase)

$2\ O_2 + NADPH \longrightarrow 2\ O_2^-$ (superoxide) $+\ NADP^+ + H^+$

(SOD)

$2\ O_2^-$ (superoxide) $+\ 2\ H+ \longrightarrow$ H_2O_2 (hydrogen peroxide) $+\ O_2$

(Catalase)

$2\ H_2O_2$ (hydrogen peroxide) $\longrightarrow 2\ H_2O + O_2$

$H_2O_2 + Fe^{2+} \longrightarrow \cdot OH^-$ (hydroxyl radical) $+\ OH^- + Fe^{3+}$ (Fenton reaction)

O_2^- (superoxide) $+\ H_2O_2 \longrightarrow$ $\cdot OH^-$ (hydroxyl radical) $+\ OH^- + O_2$ (Haber-Weiss reaction)

For many decades, oxidative stress is defined as maladaptation of cell to cope free radicals then lead to damage. There is imbalance between prooxidant and antioxidant. ROS has a role as messanger at physiological level. ROS acts like vaccine that induces defense mechanism. It should be noted individual reaction by vaccination that can cause immunity improvement, fever or unwanted results. ROS effects depend on cell types (normal, neoplasm), concentration, duration (acute, chronic) (5).

Hydrogen peroxide

The mindset about free radicals has changed since some studies at the end of 80s shown the role in physiological

mechanism. Hydrogen peroxide is formed by its spontaneous breakdown or enzymatic reaction via SOD. Hydrogen peroxide seems has many functions in physiological regulation because it is more stable and less toxic. Burke et al., 1987, found that hydrogen peroxide at micromolar levels elicits arterial pulmonary relaxation mediated by activation of guanylate cyclase. Heffetz et al., 1989, found that hydrogen peroxide potentiate tyrosine phosphorylation during insulin signaling. Burdon et al., 1989, found that hydrogen peroxide stimulates cell proliferation at low concentration. Hydrogen peroxide regulates transcriptional factors, namely AP-1, Nrf2, CREB, HSF1, HIF-1, TP-53, NF-κB, NOTCH, SP1, SREB-1. They have role in cell damage response, cell proliferation, cell differentiation, and apoptosis. Hydrogen peroxide regulates by 2 mechanisms: 1. by synthesis/degradation, 2. by controlling the activity of a pre-existence transcriptional factor (6).

Hydrogen peroxide regulates signal transduction via phosphoinositide 3-kinase (PI3K). PI3K induces phosphorylation of Akt. This results in inactivation of proapoptotic protein and activation of transcription factors that target the expression of antiapoptotic protein. This mechanism enhances cell survival. Hydrogen peroxide regulates mitogen-activated protein kinase (MAPK). Activation of MAPK promotes cell survival, growth, and differentiation. Hydrogen peroxide has paradoxical effects on NF-κB. It can activate or inhibit NF-κB activity depending on the ROS level, types of stimuli, and cell types. Moderate ROS leads to NF-κB activation, whereas high level of ROS inactivates NF-κB and leading to cell death (7).

Study by Boveris et al., 1973, showed that hyperbaric oxygen increased formation of hydrogen peroxide in pigeon heart mitochondria and rat liver mitochondria. The hydrogen peroxide level linier to increased partial pressure of oxygen. Peroxisome is rich of catalase. Heart, brain, and lungs are organs with lack of peroxisome, whereas liver and kidneys have a lot of peroxisome. The increase of hydrogen peroxide in liver less than heart can be explained by the role of catalase. It reduces hydrogen peroxide by converting to water and oxygen. Hydrogen peroxide can diffuse to cytosol and nucleus. Hydrogen peroxide induces NRF2. NRF2 is trancription factors which activates antioxidative stress response. NRF2 accumulates in nucleus leading to the induction of genes by binding to the antioxidant response element in their promoter regions. NRF2 is believed as the

regulator key in promoting endogenous antioxidants (8).

Antioxidants

Antioxidants are compunds which can cope oxidative stress by neutralizing free radicals especially ROS (oxidants). Antioxidants can be classified according to: 1. source origin, 2. solubility in water and lipid, 3. size. According to source origin, antioxidants are classified as endogenous (enzymatic) and exogenous (non-enzymatic). Endogenous antioxidants are superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), thioredoxins (TRX), peroxiredoxins (PRX). Exogenous antioxidants are vitamin C, vitamin E, polyphenol, carotenoids. Example of water-soluble antioxidants is vitamin C, and example of lipid-soluble antioxidants is vitamin E. Example of small-molecule antioxidants are vitamin C, vitamin E, carotenoids; whereas large-molecule antioxidants are SOD, catalase, and GPx (5). In the next discussion, we will discuss the endogenous antioxidants.

SOD is an enzyme that catalyze the dismutation of superoxide to be hydrogen peroxide and oxygen. In human, there are 3 forms of SOD. They are SOD-1 (cytosolic SOD; Cu,Zn SOD), SOD-2 (mitochondria SOD; MnSOD), and SOD-3 (extracellular SOD; Cu,Zn SOD). SOD-1 seems has major role to prevent oxidative stress from environmental factors. Cu,Zn SOD is dimer (32 kDa) that contains copper (Cu) and zinc (Zn). MnSOD is homotetramer (96 kDa) containing one manganese atom per subunit that cycles from Mn (III) to Mn (II) and back to Mn (II) during the two steps dismutation of superoxide (9). SOD can rapidly catalyze superoxide to be hydrogen peroxide. Lower activities of Cu,Zn SOD are often seen in tumor (10).

Catalase is a tetrameric haemin-enzyme (porphyrin heme (Fe)) consisting of four identical tetrahedrally arranges subunits of 60 kDa. Catalase catalyze hydrogen peroxide to form water and oxygen. Catalase is located in peroxisome. Lack of catalase is believed to accelerate aging and increase cell damage (9). Glutathione peroxidase (GPx) is selenium-containing peroxidase that reduce lipid peroxidation. There are 8 isoform of GPx. GPx-1 is the most abundant version, found in cytosol, and predominantly present in erythrocyte, kidney, and liver. GPx-2 and GPx-3 are extracellular enzyme, and presently in gastrointestinal and kidney. GPx-4 is predominantly in renal epithelial cells and testis. GPx-5 is expressed specifically in epididymis (9). GPx-7 and GPx-8 have been identified but still unclear.

Several studies found the effect of HBO to stimulate endogenous antioxidants. Study by Yasar et al., 2002, found HBO increased Cu,Zn SOD and GPx and decreased malondialdehyde (MDA) in acute necrotizing pancreatitis rats. MDA is the product of lipid peroxidation. Study by Matsunami et al., 2009, hyperbaric oxygen induces gene expression of GPx although not SOD and catalase in streptozotocin-induced diabetic rats. HBO induced NRF2 antioxidant pathway in order to cell survival (11, 12). HBO increased the activity of SOD and heme oxygenase-1 (HO-1) level in acetaminophen-induced renal and liver injury rats (13).

Hypoxia-inducible factor (HIF)

HIF was previously described as a transcription factor that regulates cellular metabolism and many genes under hypoxia. Recently, HIF can be regulated by nonhypoxic condition. ROS activates HIF stabilization (7). HIF consists of 2 subunit (α and β). There are 3 isoform of HIF, HIF-1, HIF-2, and HIF-3. HIF-1 is predominantly and most investigated. Under normoxia, prolyl hydroxylases degrade HIF-α. Prolyl hydroxylases have oxygen-dependent degradation domain, which binds oxygen then activates it. Under hypoxia, prolyl hydroxylases have less activitity, then HIF-α stabilize and join with HIF-β. HIF can translocate from cytosol to nucleus and activate genes transcription. HIF has roles in activation of growth factors, cellular metabolism, proliferation and differentiation (14).

It is still unclear how ROS or HBO-induced ROS can activates HIF. Possible mechanism of HBO-induced HIF is hypoxic relative. After HBO therapy finished, the condition changes from hyperoxia to normoxia. The body responds as hypoxic signal. The other hypothetical mechanism is nonhypoxia stimulus. HBO increases ROS especially hydrogen peroxide that acts to activate HIF. Growth factors, insulin, cytokines, and nitric oxide (NO) have been found that can stimulate HIF under normoxia (14). HBO induces production of growth factors. Some studies of wound healing, HBO stimulates growth factors e.g. vascular endothelial growth factor, fibroblast growth factor.

Heat Shock Proteins (HSPs)

HSPs consist of many proteins which is categorized by their molecular weight. They are HSP 20, HSP 30, HSP 40, HSP 60, HSP 70, HSP 90, HSP 110 (32, 40, 60, 70, 90, 110 kilo-daltons). HSPs are triggered by heat stimulus, but recently other stimuli can also, examples infection, inflammation, exercise, toxin exposure, radiation, starvation, hypoxia. Environmental stress including oxidative

stress (ROS) activates HSP as the defense mechanism in order to cope stress. HSP can inhibit apoptosis and maintain normal cellular homeostasis (3). HSP has 2 functions, the first is to assist in correct folding of polypeptide chain into functional protein, and the second is to assist in refolding or degradation of damaged or denatured protein after stress event. Stress stimulus like ROS results in activation of heat shock factor (HSF). HSF moves from cytosol to nucleus and attach to heat shock gene then transcription and translation of heat shock protein occur. Decreased activity of HSF and decreased expression of HSP genes may contribute cells or tissues more susceptible to oxidative stress injury (15).

Several studies found the effect of HBO in modulation of HSP. HBO induced HSP 32 expression to protect rat spinal neurons from oxidative insult and oxygen glucose deprivation injury (16). Precondition HBO induced HSP 70 to inhibit apoptosis mechanism. Expression of HSP 70 reduced ischemic injury and protected neurons and glias (14). HBO-induced ROS perhaps has benefit to heal cell by increasing HSP, but we need further studies to know the exact mechanism.

Telomere

Telomeres are specialized DNA structures located at the terminal ends of the chromosomes. The functions of telomeres are to maintain genome stability, cell survival and senescence. Telomerases are enzyme which improve telomere length after mitotic division. Telomere shortening can lead to senescence and apoptosis. Hayflick limit is telomere shortening after approximately 50 times mitosis. Telomere length has been associated with degenerative diseases, severity of oxidative stress, and aging process. Telomere has been used as parameter of life span. The several studies found that oxidative stress caused telomere shortening and deterioration of telomerase capacity. Exercise and nutrition increased telomerase activity (17).

Study by Mishra et al., 2016, found that ROS was beneficial to maintain sperm telomere length. Mild oxidative stress increased telomere lengthening whereas severe oxidative stress decreased it (18). This finding need further investigations. If we look at exercise that it also can elevate ROS but exercise has benefit in telomere lengthening by increasing telomerase activity. It is still unclear how ROS can regulate telomere. HBO elevates ROS in the body but improve health and inhibit deterioration in aging. We still do not have

evidences the role and mechanism of HBO-induced ROS in regulating telomere and telomerase.

CONCLUSIONS

HBO-induced ROS has beneficial effects to maintain cell survival. The mechanism is probably via hydrogen peroxide that has roles in cell proliferation, differentiation, and survival. HBO-induced ROS increases endogenous antioxidants that can remove and reverse oxidative stress. HBO activates HIF via hypoxia relative mechanism or directly mediated by ROS. HBO stimulates HSP that can improve defense mechanism and cell survival. HBO probably influences telomere and telomerase but this teoritical mechanism needs further evidences.

Abbrevations

AP-1 = activator protein – 1

Nrf2 = nuclear factor-erythroid 2 p45-related factor 2

CREB = cAMP response element-binding protein

HSF1 = heat shock factor 1

HIF-1 = hypoxia inducible factor -1

TP-53 = tumor protein – 53

NF-κB = nuclear factor kappa bheta

SP1 = specificity protein 1

SREB-1= sterol regulatory element binding protein - 1

HBO = hyperbaric oxygen

ROS = reactive oxygen species

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Table 1. Classes of ROS and their properties (19)

Radical	Structure	Reactivity	Half-life	Production/localization	Diffusion	Targets
Hydroxyl radical	$\cdot OH^-$	High	10^{-9} second	Mitochondria, phagosome, endoplasmic reticulum	Highly localized where is produced	Any cell components
Superoxide	$\cdot O_2^-$	Low	1-15 minutes	Mitochondria, cytosol, endoplasmic reticulum, peroxisome, plasma membrane, lysosome	Localized, it can diffuse through an anion channel	Fe-S centers, nitric oxide
Hydrogen peroxide	H_2O_2	Moderate, reversible	Hours to days	Mitochondria, cytosol, endoplasmic reticulum, peroxisome, plasma membrane, lysosome	Diffuse, it can travel through aquaporins	Fe-S, cysteine residues