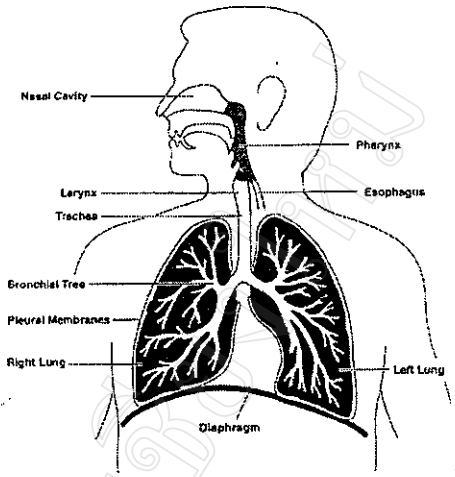
CHAPTER I

LITERATURE REVIEW

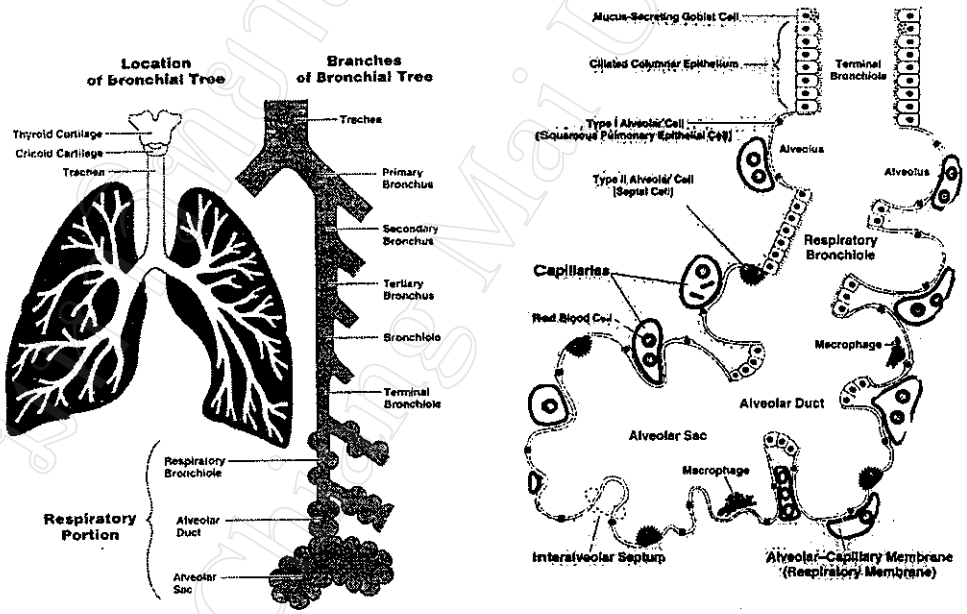
PULMONARY SYSTEM

In the summary of pulmonary system, a part of anatomy and physiology is well known knowledge. Anatomy of the respiration system is composed of sequence structures, nose, pharynx, larynx, bronchial tree, and alveolar. (Figure.1 A.) The physiology of respiration system is effective ventilation and oxygenation. Bronchial tree is the main part of the air conduction and regulation airflow from atmosphere into the lung (Figure. 1B). Deeply part is alveolus portion (Figure.1C), the essential component are alveolar type I and II cells that prevent the leakage of tissue fluid into the alveolar air space and produce surfactant for sufficient lung compliance respectively. Pulmonary interstitium is the space interposed between the air space epithelium, the vascular endothelium and the pleural mesothelium. In the interstitium mesenchymal cell compose of various cells, fibroblasts, myofibroblasts, pericytes and smooth muscle cells (Bastian, 1994)

Connective tissue in the lung is found throughout the lung from the largest conducting airways to alveoli in the lung parenchyma. Main components in the lung compose of collagens, elastin and proteoglycan (Figure 2). Elastin, a protein with rubber-like qualities, giving lung its high compliance and elasticity. Collagen, with a more rigid structure, provides the architectural framework but impose limits



(A)



(B) (C)

Figure 1. Show the anatomy of pulmonary structure; component of respiratory system (A), bronchial tree (B), and alveolar sac (C). (Bastian, 1994)

but impose limits to expansion. In this way, during a complete breathing cycle, the lung undergoes marked expansion, generating large recoil forces, but maintains its fine structure with a close opposition between epithelial and endothelial cells. Collagen and elastin together represent about one-fifth of the dry weight of the human lung. In addition, proteoglycans are present in a concentration about one-thirth that of collagen, and they may play a critical role in lung function, particularly in relation to fluid transport.

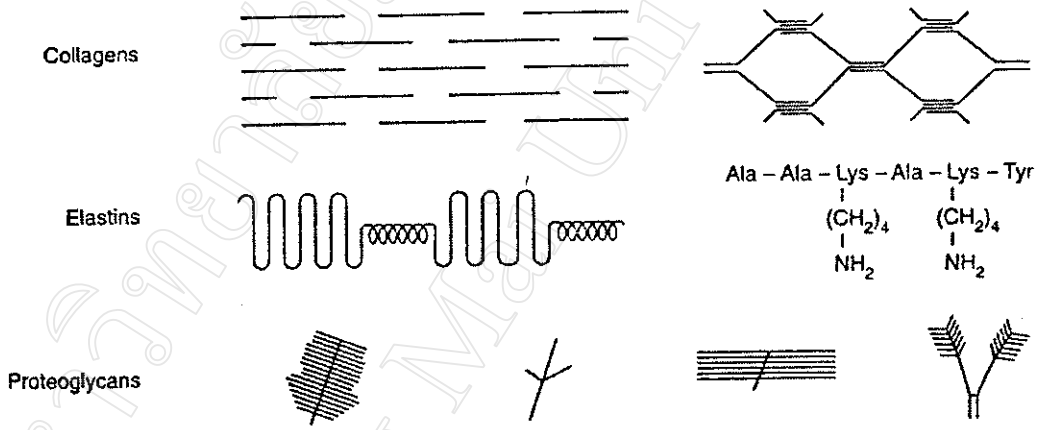


Figure 2. Component of the extracellular matrix. (Ala = alanine, Lys = lysine, Tyr = tyrosine). (Laurent, 1995)

Fibroblasts are engaged in active fibrogenesis and secrete collagen, proteoglycans and fibronectin that are important constituents of the connective tissue matrix (Figure 3) . Especially proteoglycan in which compartment in the alveolar wall are a class of complex macromolecules comprised of a protein core are associated with large sugar called " glycosaminoglycans". The major proteoglycans are heparan sulfate, chondroitin sulfate, and dermatan sulfate, including hyaluronic acid (HA). (Figure 6)

FREE RADICAL

The oxyradical of free radicals are produced continuous in the normal cell; controlled by variety of antioxidants that developed to protect itself. There have many sources of free radical products such as mitochondria electron transport chain , eicosanoid metabolism, xantine oxidase function, respiratory burst, nitric oxide synthesis function, and auto-oxidation (Zimmerman, et al., 1995). Free radicals are molecules that contain one or more unpaired electrons. Molecular oxygen (O_2) itself is a diradical with an unpaired electron in each of its two outer orbital. Various enzymes, particularly oxidase, are designed to lower this activation energy. Within the mitochondria electron transport chain, completed controlled reduction of O_2 occurs according to the reaction (Equation 1)

$$O_2 + 4H^+ + 4e^- \longrightarrow 2\,H_2O \qquad (1)$$

The terminal event in a series of couple redox reactions allows efficient, unstable energy production in the form of adenosine triphosphate (ATP), with the O_2 used as the ultimate electron acceptor. Incomplete reduction of O_2 can also occur and may generate a family of highly reactive oxygen species as summarized: (Equation 2-5)

$$O_2 + e^- \longrightarrow O_2^{o-} \qquad (2)$$

$$O_2^{o-} + e^- + 2H^+ \longrightarrow H_2O_2 \qquad (3)$$

$$H_2O_2 + e^- + 2H^+ \longrightarrow HO^o + H_2O \qquad (4)$$

$$HO + e^- + 2H^+ \longrightarrow H_2O \qquad (5)$$

Oxygen radicals have been demonstrated to mediate injury in the four major classes of cellular macromolecules as carbohydrate, lipids, proteins, and nucleic acids. Molecular alternation may be reflected in both structural and functional consequences.

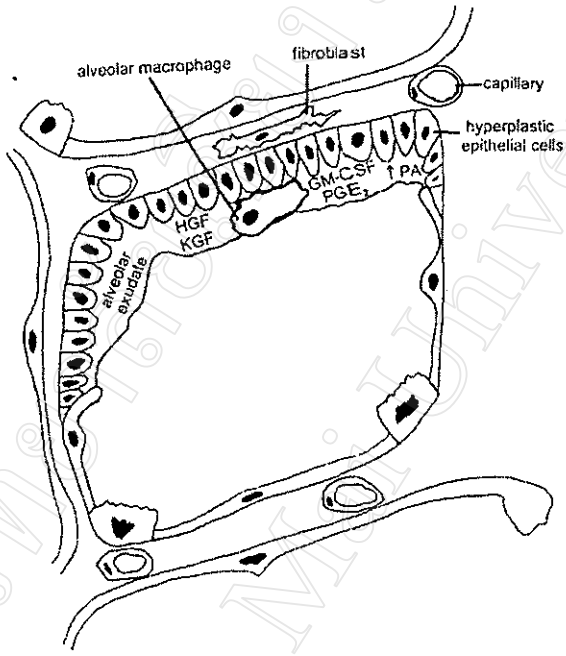


Figure 3. The alveolar cross section shows the component in the alveolar sac, alveolar macrophage, capillary, hepatocyte growth factor (HGF), GM-CSF, PGE2, and fibroblast. (Toews, 1999)

Protein is denatured by oxyradicals results in the increased susceptibility to proteolysis. Amino acids in the proteins, which are influenced, are tryptophan, tyrosine, histidine, cysteine, and methionine. Change in primary structure in each amino acid may result in significant changes in secondary, tertiary, and quaternary structures. For example; superoxide radical reacts to actin cytoskeleton by oxidation, including uncouple energy protein on mitochondria or band 3 protein on the red

blood cells. So that mitochondria can not regenerate energy for many activities and carrying oxygen to the normal cells.

Nucleic acid, probably the most injury resulting from cellular oxidative stress relate to the alternation of nucleic acid, it would be expected to not only alter the contemporary synthetic activity of the cell but also have pronounced effects on subsequent growth, differentiation, replication, and repairing. Free radicals are associated with DNA strand breakage as well as enhanced sister chromatid exchange.

Lipid cell membrane is composed of protein and lipid compartment mainly in lipid bilayer model. Polyunsaturated fatty acid (PUFAs) represents a large-mass highly susceptible target for oxyradicals attachment. Lipid peroxidation is commonly found of the degradation by free radicals with three processes (Halliwell, et al., 1989). Initiation process is initiated by the reaction of polyunsaturated fatty acid (PUFAs) with free radical which produces numerous fatty peroxyl readicals. Propagation, which is an autocatalytic process, is activated by another free radical specie, including the perhydroxyl radicals ($HOO^{\bullet}$) or peroxyl radicals ($ROO^{\bullet}$). Especially the carbon radicals can react with oxygen to form another peroxyl radical and also stimulate the chain reaction of lipid peroxidation continuously. Peroxidation of arachidonic acid that lies on the membrane can activate phospholipase C (PLC) to break phosphatidylinositol down to inositolphosphate (IP) and diacylglycerol (DAG) (Bottje, et al., 1998), giving six lipid hydroperoxides as well as cyclic peroxides and other products.

LUNG INFECTION, FREE RADICALS AND LUNG DAMAGES

Lung infection with virus or bacteria is mostly ofound in the neonatal or pediatric patients in the hospitals and its various severity of lung tissue depends on the type and quantity of microorganisms. (Niederman, et al., 1994) Respiratory tract

infection is divided into two types: upper airway infection as pharyngitis, sinusitis, otitis media, epiglottis, bronchitis and tracheobronchitis; and lower airway infection as pneumonia and cystic fibrosis. (Nelson, et al., 1995). Infection stimulates inflammatory process normally within 2 hours and mostly still affects within 4 to 6 hours later (Carey, et al., 1992; Haslett, et al., 1995; Reynolds, et al., 1997). Infection induces the tissue injury involves in many pathways: as following as; mitochondria damage, oxidant enzyme, nitric oxide synthase, phagocyte recruitment and iron releases that produce free radicals such as superoxide, hypochorus radical, nitrogen species , hydroxyl radicals, and nitric oxides (Halliwell, et al., 1989A).

Stimulation of macrophages can produce superoxide radicals. Despite lack of myeloperoxide, they contain catalase and gluathione peroxidase, when both can catalyze the bacteria be the affect of hypochlorous acid (HOCL). Hydrogen peroxide , peroxidase and HOCL can kill bacteria directly or by formation of free halide radicals or aldehydes (Figure 4) (Spector, et al., 1999).

The sequence process of inflammation shows increasing neutrophill (Adams, et al., 1993; Downey, et al., 1999) including T and B-lymphocytes that secrete pro-inflammatory mediators such as leukotriene, tumor necrotic factor (TNF), interleukin-1 (IL-1), interleukine-8 (IL-8). Some evidents show that neutrophil accumulated in the lungs of infants with respiratory distress is an important contributor to the pathogenesis of chronic lung diseases. The pro-inflammatory mediators can give specific free radicals; for instances, mitochondria produce superoxide radicals and hydrogen peroxide, white blood cells produce hypochlorous acid, and membrane of the cells show peroxyl radicals (Buss, et al., 2000).

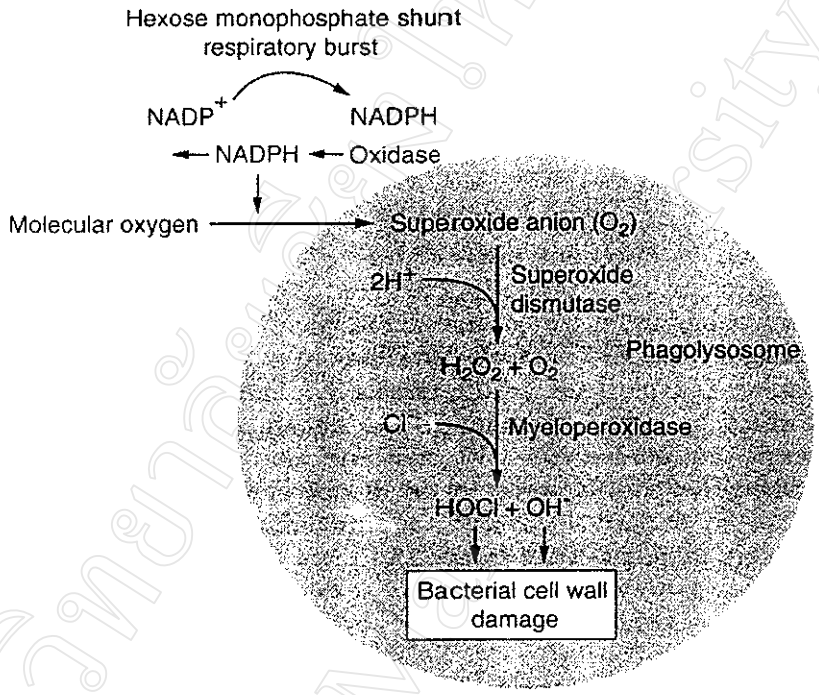


Figure 4. Mechanism if oxygen-dependent bacterial killing in a phagocyte ($NADP^+$ = Nicotinamide adenine diphosphate, H_2O_2 = hydrogen peroxide, Cl^- = chloride ion, OH° = hydroxyl radicals, HOCL = hypochloroous radical) (Spector 1999)

Free radicals are undoubtedly involved in virtually every aspect of human disease and probably in many aspects of normal homeostatic cellular physiology. The production of various free radicals species by phagocytes represents an essential aspect of immunity, whereas absent free radical production is associated with chronic infection. Under normal condition, intracellular superoxide and hydrogen peroxide radicals concentration are kept at a low levels about 10 pM and 1-1000 nM respectively. They are produced and controlled by cytosolic and mitochondrial

superoxide dismutase (SOD), cytosolic glutathione oxidase, peroxisomal catalase and several nonenzymatic antioxidants (Hadad, et al., 2000).

Especially superoxide radicals are released by macrophage function and can activate NADP-oxidase and cytochrome b reductases that can activate the catabolism pathway of oxygen and NADPH to superoxide radial and $NADP^+$. Hydrogen peroxides (H_2O_2) is released from polymorphonuclear leukocyte (PMNs), that also destroy the membrane activates lipid peroxidation and also directly affects on DNA strand. (Equation 6-7)

$$O_2 + NADPH, H^+ \xrightarrow{\text{NADPH-oxidase}} O_2^- + NADP^+ + H^+ \quad (6)$$

$$O_2 + e^- \xrightarrow{\text{Cytochrome b reductase}} O_2^- \quad (7)$$

An aggressive free radical is also promoted from external factors such as prematurity, fever, severity and chronic stage of infection, including hypoxia. Red blood cell carries oxygen with heme, which can activate the fenton reaction and catalyse hydrogen peroxide to hydroxyl radicals. The means of signaling pathway of inflammation from infection to produce free radicals as well as provoke hypoxia in the cell can stimulate free radicals that can finally destroy the tissue. Acute lung injury from free radicals is the most likely in both capillary endothelial and alveolar epithelial injuries. In the other way lung injury may be stimulated by hyperoxic condition, 100% oxygen for 8 to 12 hours or hypoxemia, poor ventilation and circulation.

Lipid peroxidation

From the study shows the relationship between lipid peroxidation and myeloperoxidase activity in tracheal aspirates of preterm infants (Adams, et al., 1993). Lipid peroxidation has been found in relation with lung injury (Demling, et al., 1989). Peroxidation of lipid, especially polyunsaturated fatty acids (PUFAs) on the cell membrane by free radicals and lipid peroxide products have shown in three steps.

Initiation step: Conjugated diene bonds formation is generated by attraction of hydrogen atom. Single free radical ($R^{\bullet}$) removes an H atom from a lipid molecule (LH), so give lipid radical ($L^{\bullet}$). The reaction is shown in equation 8.

$$LH + R^{\bullet} \longrightarrow L^{\bullet} + RH \qquad (8)$$

Propagation step: The free radical from the initiation step interacts with an oxygen molecule. To produce lipid peroxyl radical ($LOO^{\bullet}$). Formed lipid peroxyl redical subsequently reacts with lipid molecule (LH) to generate lipid hydroperoxide (LOOH) and lipid readical as shown in equation 9 and 10.

$$L^{\bullet} + O_2 \longrightarrow LOO^{\bullet} \qquad (9)$$

$$LOO^{\bullet} + LH \longrightarrow LOOH + L^{\bullet} \qquad (10)$$

Terminal step: Chain reaction can be terminated by antioxidants (AH) such as Vit E or GSH directly that can change lipid peroxyl radical to lipid hydroperoxide as shown the equation 11 and 12 respectively.

$$LOO^{\bullet} + VitE \longrightarrow LOOH + Vit\ E^{\bullet} \qquad (11)$$

$$LOO^{\bullet} + GSH \longrightarrow LOO\text{-}SG + GS^{\bullet} \qquad (12)$$

Ineffective antioxidant capacity induces more lipid peroxyl radical (LOO•) and PUFAs oxidation that causes the formation of hydroperoxides with conjugates dienes. Hydroperoxide molecules can undergo a formation of hydrocarbon gasses as pentane, aldehyde compounds, MDA.

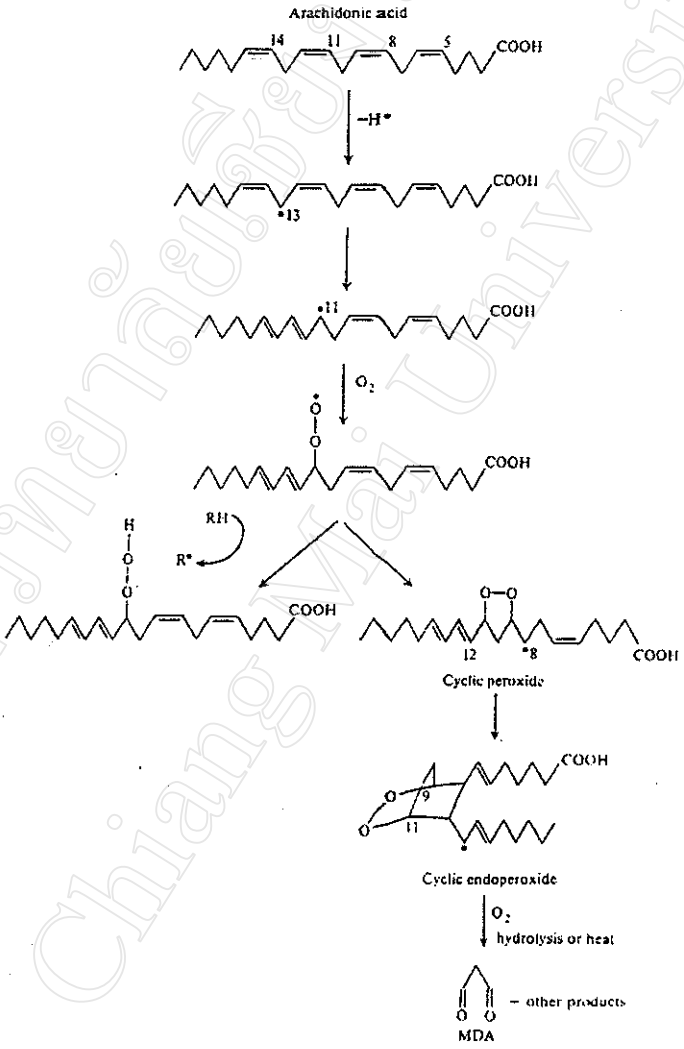


Figure 5. Proposed mechanism for formation of lipid peroxidation from arachidonic acid. (MDA = malondialdehyde) (Halliwell, et al., 1989B)

Hyaluronic acid (HA)

In the bronchial tree, bronchial cartilage is composed of various tissues and another components. Proteoglycan is the most abundant in bronchial cartilage and in lung parenchyma (Figure 6). Recent studies have suggested that it regulates the fibrillogenesis of collagen and elastin. HA is a major of the water absorber in the extracellular matrix, where also influences the mobility of cells and other cellular function. HA is synthesized in the plasma membrane of most mesenchymal cells, especially by activated fibroblasts. It is capable absorbing water so that promotes the alveolar distension whiling expansion. High concentrations of HA in the lung tissue are found during remolding of tissue in the course of normal development as well as in diseases. Inflammation in the lung from oxygen-induced and free radical stimulate the fibroblast cells to produce HA (Johnsson, et al., 1998; Elias, et al., 1988).

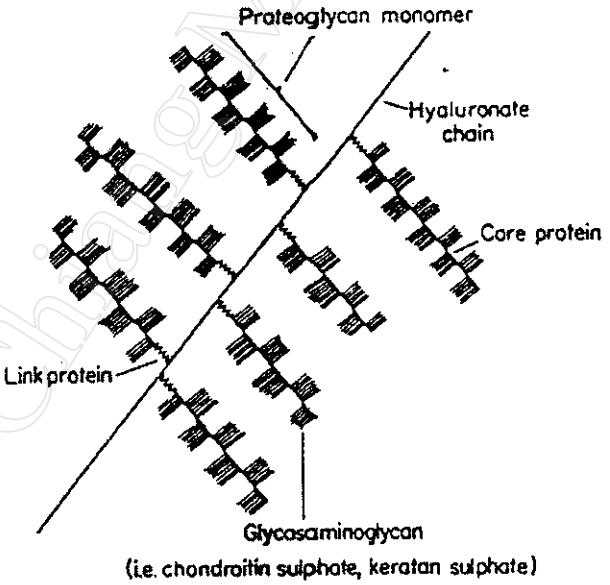


Figure 6. Hyaluronic acid (HA) structure. (Laurent, 1995B)

HA is stimulated by pro-inflammatory mediators from macrophages and directs to the neutrophil in the capillary (Figure 7). Free radicals in the cells may destroy the interstitium of alveolar cell, and increasing the hyaluronic acid (HA) concentration in the bronchoalveolar larvage (BAL), that are found in parenchyma disease (Hallgren, et al., 1989).

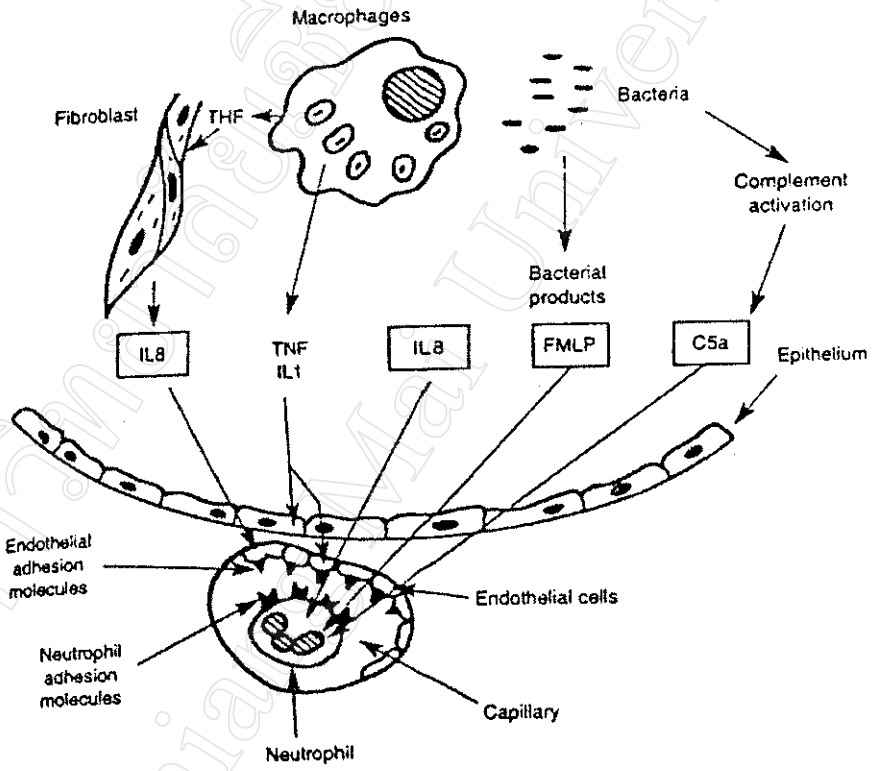


Figure 7. The early events in the evolution of lung inflammation; attraction eutrophils to the lung in the initial state of acute inflammation, Fibroblast is one of the cell that stimulated and induced the secreted hyaluronic acid. (IL= interleukin, TNF= tumor necrotic factor) (Haslett, 1995)

ANTIOXIDANTS IN THE LUNG

Antioxidants in the lung have two types mainly, primiary antioxidant is a group of enzymes and secondary antioxidant is a group of chemical substances in the body such as Vit E, beta-carotene, uric acid, ascorbic acid, and glutathione. Both antioxidants cooperate to reduce the intra-cellular and extra-cellular toxicity (Frank, et al., 2000). Primary antioxidant (Table 1) is a group of enzymes that can reduce or changes the intermediates of free radicals; for example, superoxide dismutase (SOD) (Russell, 1994), catalase, glutathione peroxidase, and glutathione reductase (Repine, et al., 1997). Secondary antioxidants (Table 2) are local found in extracellular compartments such as glutatione (Mesite, et al., 1983; MacNee, 1997; Buhl, et al., 1994), beta-carotene (Grievink, et al., 1998; Malone, 1991), bilirubin, albumin, alpha-tocopherol (Vit E), ascorbic acid (Vit C), uric acid (Schrod, et al., 1997), and synthesized substances as N-acetylcysteine (Konrad, et al., 1995), N-acystenyl (NAL) (Gillssen, et al., 1997), dihydrolipoic acid (DHLA) (Biewenga, et al., 1998), and oleic acid (Hart, et al., 1997).

GLUTATHIONE

Glutathione (L-glutamyl-L-cysteinyl-glycine; GSH) is a tripeptides and widely distributed in animals, plants, and microorganisms. GSH is found in the intracellular, low concentration about 2 μM in human plasma (Anderson, 1997), and also found in extracellular parts as in nasal lining fluid at about 40 μM, and in epithelial lung fluid (ELF) at about 100 μM, and including red blood cells about 2 mM (Lkegami, et al., 1994; Mesiter, et al., 1976). Recent identification of thiol-specific protector also play a similar role in some cells (e.g., erythrocytes). Total reactive thiols of normal human erythrocyte are composed of the thiol-groups of hemoglobin (80 to 85 %), GSH (10 to 15%) and membrane protein (5 %).

Table 1. Antioxidant defense systems with enzymes and their roles in prevention of cell damage by cytotoxic O_2 species: (Frank, 2000)

Reactive O_2 species	Antioxidant enzymes	Cell components attacked by reactive O_2 species
O_2^- (Superoxide)	SOD $2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$	Lipid: peroxidation of unsaturated fatty acids in cell membranes.
H_2O_2 (hydrogen peroxide)	Catalase $2H_2O_2 \longrightarrow 2H_2O + O_2$	Protein: oxidation of sulfhydryl-containing enzymes
ROO• (peroxyl radicals)	GP $2\ ROO^\bullet + 2H^+ \longrightarrow O_2 + 2\ ROH$	Carbohydrates: depolymerization of polysaccharides
$^1O_2$ (Singlet oxygen)	$^1O_2$ (scavenged by beta-carotene)	Nucleic acids: base hydroxylation, cross-linked, DNA strand scission
OH• (hydroxyl radical)	OH• (scavenged by vitamin E (?), GSH (?), Ascorbate (?)	(Also, inhibition, nucleotide, fatty acid bio synthesis)

Table 2. Antioxidant defense systems with substance and their roles in the prevention of cell damage by Cytotoxic O_2 Species. (Frank, 2000)

Principle Component	Defense mechanism
Lipid Soluble	
Vitamin E (tocopherol)	Reduces chain-reaction lipid peroxidation in cell membranes, may directly convert O_2^- and OH radicals to less reactive forms.
Beta-carotene (precursor or Vitamin A)	Scavengers O_2^- and $^1O_2$; may react directly with peroxyl radicals.
Water Soluble	
Vitamin C (Ascrobate)	Directly scavenger O_2^- and $OH^\bullet$ radicals; contributes to regeneration (reduction) of oxidized vitamin E.
Glutathione (GSH)	Substrate for glutathione peroxidase; may react directly with O_2^-. $OH^\bullet$ radicals and may also regenerate oxidized vitamin E

Characteristic structure of GSH composed of sulfhydryl (SH) group and γ- glutamyl linkage as following.

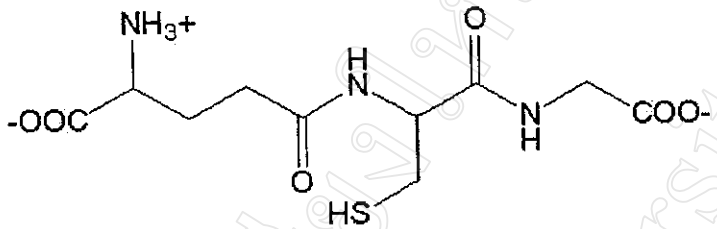


Figure 8. Structure of glutathione (GSH), (L-γ - glutamyl -L-cysteinyl-glycine)

Glutathione is synthesized intracellularly by the consecutive actions of glutamylcysteine and GSH synthetase (Anderson, et al., 1997). (Equation 13-14)

$$\text{L-glutamate + L-cysteine + ATP} \xrightarrow{\text{glutamylcysteine synthetase}} \text{L-}\gamma\text{-glutamyl-L-cysteine + ADP + Pi} \quad (13)$$

$$\text{L-}\gamma\text{-glutamyl-L-cysteine + glycine + ATP} \xrightarrow{\text{GSH synthetase}} \text{GSH + ADP + Pi} \quad (14)$$

Significant function of GSH are protecting cell membrane or protein by maintaining essential SH group, interacting with peroxides or free radicals, transporting amino acid across membranes, or to be a coenzymes for certain enzymes (i.e., glycoxylase, maleylacetoacetate isomerase, protaglandin endoperoxide isomerase) (Ziegler, 1985).

Important function that is a new theory, GSH is an extracellular antioxidant for free radicals or reactive metabolites, and conjugates whith toxicants (Figure 9).

Glutathione is an extracellular antioxidant for free radicals or reactive metabolites, and conjugates toxicants (Figure 9). Alternatively, glutathione may donate a hydrogen atom to a free radical intermediate and be converted into a gluathione radical (thiyl radicals), which may subsequently react with another glutathione radical to form GSSG or abstract a hydrogen atom from other substances to form new radicals and GSH. Thus, the result of these types of reactions may be the oxidation of glutathione. Under normal conditions glutathione reductase, an enzyme that requires NADPH (reducing equivalents) generated from glucose-6-phosphate dehydrogenase can reduce one molecule of oxidize (GSSG) to form reduce GSH. (Equation 15)

$$GSSG + 2\,NADPH \xrightarrow{\text{reductase}} 2\,GSH + 2\,NADP + H^{+} \quad (15)$$

Two forms of glutathione peroxidase (PGX) have been demonstrated. One form requires selenium as a cofactor and is capable of reducing both lipid peroxides and hydrogen peroxide, where as the non-selenium enzyme only hydrogen peroxide. PGX activity is high in the liver and erythrocytes, with intermediate levels found in the heart and lungs.

GSH has an protective effect in vivo because it is a substrate for glutathione peroxidase as well as being able to react directly with the SH-group of membrane protein and with various aldehydes produced during lipid peroxidation (Halliwell, et al., 1989B) such as lipid peroxides ($LOO^{\bullet}$) (Equation 12) or inhibition the final product, MDA, hexanal, and 4-hydroxynonenal (HNE).

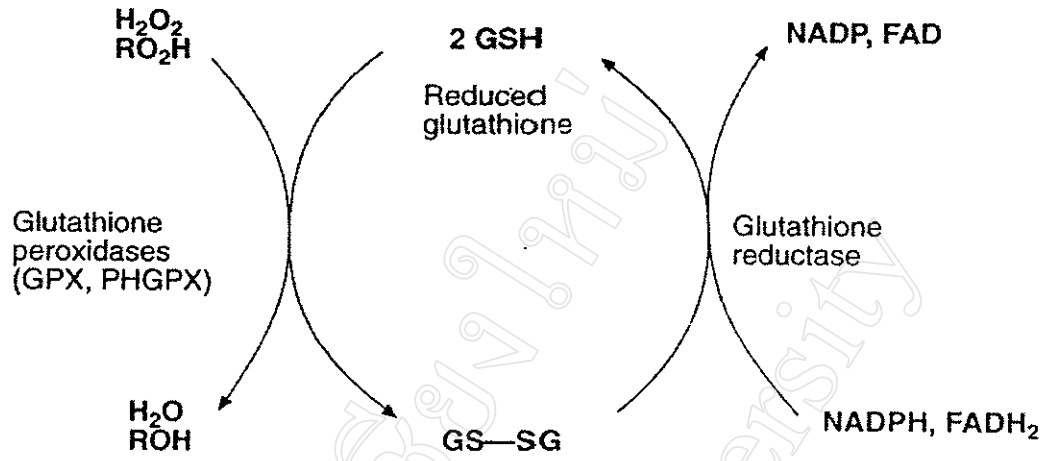


Figure 9. Show the GSH can detoxify the free radical (ROOH) and hydrogen peroxide (H_2O_2) and recycle GSH by glutathione reductase and NADPH or $FADH_2$ (NADPH or $NADP^+$ = reduced or oxidized nicotinamide dinucleotide phosphate, GSH = reduced glutathione, GS-SG = oxidized glutathione, ROOH = organic peroxides) (Parke, 1999)

ALPHA-TOCOPHEROL (Vitamin E)

The alpha-tocopherol represents the primary chain-breaking antioxidant in hydrophobic environments as a scavenger of peroxyl radicals that may be from lung infection (Cetinkale, 1997). Its structure is characterized by a chroman ring that facilitates the radical trapping capacity of the molecule and a long aliphatic tail allows it to attach in lipid membrane. Vit E can secreted by type II pneumocytes for surfactant lipids (Rustow, et al., 1993)

α-tocopherol

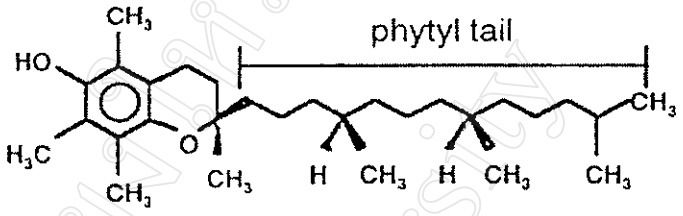


Figure 10. Structure of alpha-tocopherol (Traber, et al., 1999)

Alpha-Tocopherol (Toch) and tocotrienols inhibit lipid peroxidation largely because they scavenge lipid peroxide ($LOO^{\bullet}$) radicals much faster than these radicals can react with adjacent fatty acid side-chains with membrane protein. (Equation 17)

$$TocH + LOO^{\bullet} \longrightarrow Toc^{\bullet} + LOOH \qquad (17)$$

One molecule of alpha-tocopherol is capable of terminating two peroxidation chains. Principle of the above reaction includes eight alpha-substitited tocopherones and epoxy (hydroperoxy) tocopherones: the former readily hydrolyzes to tocopheryl quinone and the latter to epoxyquinones. In the experiment of vitamin E supplement in toxic-induced rats compares with control rats are found that less lipid peroxidation (Kovacheva, et al., 1996). (Figure 11)

In addition to ascorbic acid, GSH may be involved in the regeneration of vitamin E (Halliwell, et al., 1989A) . The view is promoted by the observation of the protective effects of GSH against lipid peroxidation in various in vitro system. Mention should be made of the key of glutathione (GSH), which is responsible for

maintain a reduced sulfhydryl intracellular redox state from reduce free radicals (Zimmerman, 1995). In the process, glutathione sulfhydryl is metabolized to the corresponding disulfide and then subsequently reduced back to the sulfhydryl through the action of glutathione reductase by using NADPH reducing equivalents.

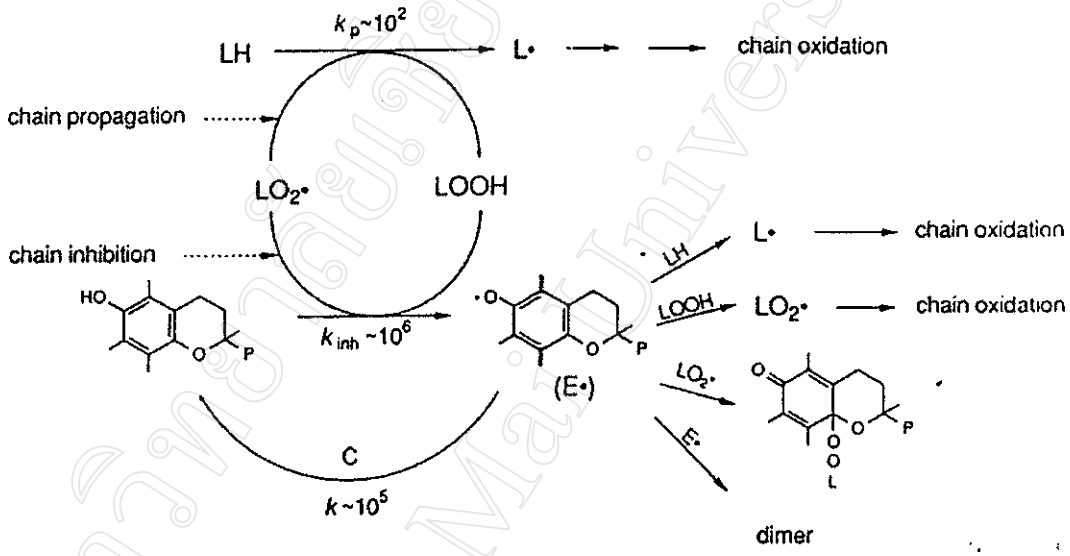


Figure 11. Inhibition of lipid peoxidation by alpha-tocopherol. LH, lipid; $L^{\bullet}$, lipid radical; $LOO^{\bullet}$, lipid peroxyl radical; LOH, lipid hydroperoxides; C, vitamin C; P, phytyl side chain $C_{13}H_{27}$; $E^{\bullet}$, alpha-tocoperhol radical; $K^{\bullet}$, rate peroxyl radial (Niki, 1996)

In conclusion, the effects of GSH and alpha-tocopherol can protect free radical-induced lipid peroxidation tissue damage (Figure 12) (Buss, et al., 2000; Chow, 1991). It can detoxify the LOOH and LOO• radicals from chemicals radiation and oxidant by preventing effects of LOO• from damaging the membrane and DNA in the cells.

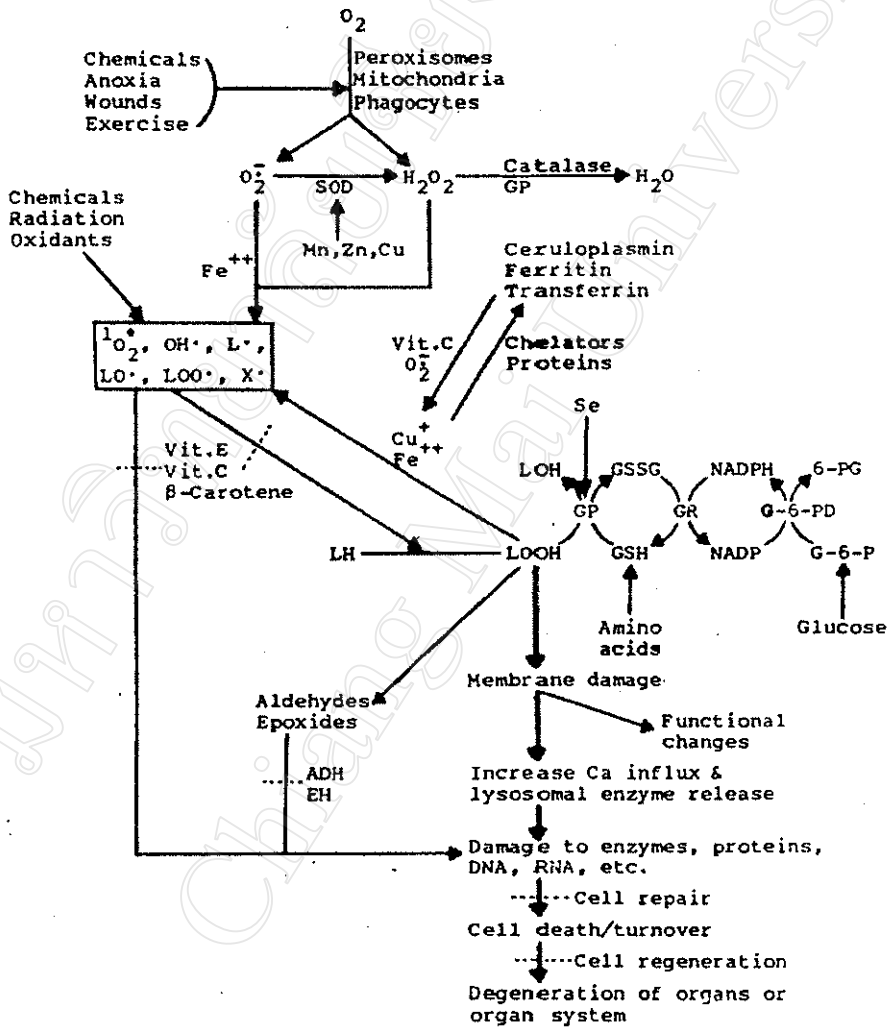


Figure 12. Scheme of free radical-induced lipid peroxidation tissue damage and antioxidants defense. (Chow, 1991)

Neonate with lung infection and Oxidative stress

During January 1997-November 1999, about 50 of 207 neonates patients at the acute care unit in Maharaj Nakorn Chiang Mai Hospital, died with lung infection. Special diseases in the pulmonary system or chronic lung diseases are bronchopulmonary dysphasia (BPD) and idiopathic respiratory distress syndrome (IRDS). (Coalson, 2000; Smith, et al., 2000) High potential of free radicals are the most common found related with prematurity, hyperoxia and barotrauma from ventilators or inflammatory auto-injury (Bruce, et al., 1992). Animal model has shown that hyperoxic in the lung exposure to 100% oxygen, lung parenchymal microsomes displays increased levels of lipid peroxidation concomitant with increases in lung endogenous phospholipases, leukotriene B4 and neutrophil.

MDA is a product of lipid peroxidation that seems related with the lung injury. There are reference values obtained for plasma lipid peroxides from the thiobarbituric acid reactive substance (TBARs) assay and shown ranged of age in years such as; less than 10 year about 2.46 to 1.26 μM in healthy boys and 2.56 to 1.6 μM in healthy girls (Yagi, 1998). In clinical studies have found that the high MDA/cholesterol ratio in the cystic fibrosis (McGrath, et al., 1999), The high MDA concentration is inversely correlation with plasma of Vit E in the patients who suffers from septic shock and secondary organ dysfunction. (Goode, et al., 1995).

From the previous basic of HA structure and function, the same studies show the good relation between lung injury and HA in blood and bronchoalveolar lavage (BAL). (Ishibashi, et al., 1995).

A few data show the significantly high HA level in the serum and BAL of adult respiratory distress syndrome (ARDS) (Hallgren, et al., 1989)) and in sarcoidosis patients (Bjermer, et al., 1987).

Antioxidant, glutathione (GSH) is present at a very low concentration that found in preterms and diseases. In healthy term newborns are also shown high oxidative stress, but no difference GSH concentration in healthy adults (66.4±12.5 and 64.9±9.33 mg/RBC dl respectively) (Metsvaht, et al., 1999).

In the study of premature infants with chronic lung disease (CLD) show low concentration of glutathione in BALF about 1.1 μM (0.5-3.1 μM) that compares with that in non CLD, 4.7 μM (0.2-16.8 μM) (Grigg, et al., 1993).

Premature infants are deficient in vitamin E, as with other aspects of antioxidants defense. High oxidative stress from lung infection is correlated with a low stability of red blood cell and can be protected by the supplement with vitamin E. Low of antioxidants in the lung have shown and studied in another patients, such as in adult respiratory distress syndrome (ARDS) and infection (Grigg, et al., 1993; Pacht, et al., 1991). The quantity of Vit E is determined in the blood; various concentration up to ages, as children 2 to 12 years old, 18 μM in term infants, and 9 μM in premature infants. The previous study shows the level of alpha-tocopherol in preterm and in healthy adults are 2-3 mg/L and 5-20 mg/L respectively (Wewan, et al., 1993).

LUNG INJURY

Pulmonary function can be detected with blood gases in arterial and venous blood, then can be interpreted directly as an improvement or deterioration in pulmonary function. The data is composed of oxygen pressure in artery (PaO_2), carbon dioxide pressure in artery ($PaCO_2$), pH, base excess (BE), total carbon dioxide (tCO_2) and oxygen saturation. Definition of lung injury is used widely in clinical application; especially, for studying in acute respiratory distress syndrome (ARDS) patients (Murry, et al., 1988).

The first two parts of the scoring system in corporate information that is available on every patients; the second of two parts include data that can be obtained from mechanically ventilated patients. The extent of roentgenographic densities provides an assessment of the presence and severity of increased permeability pulmonary edema, the chief consequence of acute pulmonary parenchyma injury, and of the subsequent resolution or organization of the process. Gas exchange abnormalities, may correlate poorly with the extent of pulmonary edema and thus are an additional marker of the presence and severity of pulmonary fractional oxygen tension divided by the fractional concentration of inspirated oxygen (FIO_2).

Previously to account for changing levels of ventilation and arterial carbon dioxide tension, by calculate alveolar oxygen tension (PAO_2) instead of using the FIO_2, and report the PaO_2/PAO_2 ratio. However, the PaO_2/FIO_2 ratio provides a similar assessment of gas exchange, and is more easily calculated from information routinely available in patients' charts. The added values for positive end expiratory-pressure (PEEP) takes into account by the facts that some patients are already being ventilated with PEEP at the time their pulmonary parenchyma injury occurs, and that, in this setting, PEEP usually shows a beneficial effects on gas exchange,. No score is assigned for PEEP value up to 5 cmH_2O because theses levels are often used for ventilating patients whose lung are normal.

The components and individual values of the lung injury score have 3 levels, no lung injury, mild to moderate lung injury, and severe lung injury as following;

1. chest roentgenogram score

No alveolar consolidation

Alveolar consolidation confined to 1 quadrant

Alveolar consolidation confined to 2 quadrants

Alveolar consolidation confined to 3 quadrants

Alveolar consolidation confined to all 4 quadrants

2. Hypoxemia score

PaO_2/FiO_2	> 300
PaO_2/FiO_2	225-299
PaO_2/FiO_2	175-224
PaO_2/FiO_2	100-174
PaO_2/FiO_2	< 100

3. PEEP score (whem ventilated)

PEEP	$\leq$ 2 cmH_2O
PEEP	6-8 cmH_2O
PEEP	9-11 cmH_2O
PEEP	12-14 cmH_2O
PEEP	$\geq$ 15 cmH_2O

4. Respiratory system compliance score (when available)

Compliance	$\geq$ 80 ml/ cmH_2O
Compliance	60-79 ml/ cmH_2O
Compliance	40-59 ml/ cmH_2O
Compliance	20-39 ml/ cmH_2O
Compliance	$\leq$ 19 ml/ cmH_2O

Calculated score is obtained by dividing the aggregate sum by the number of component. The results of score are divided into three classes; no lung injury (score 0), mild to moderate lung injury (score 0.1-2.5) and last severe lung injury (score more than 2.5). A few analytically practical studies with acute lung injury score are used in clinical study, such as studying the physiologic changes in placing pediatric patients with acute lung injury (ALI) prone for 20 hour per day during acute phase of their illness with bilateral parenchyma disease. Sometime the PaO_2/fraction of inspired oxygen (FIO_2) ratio is used to be a marker of lung inury only (Curley, et al., 2000). For example, studying the effect of extracorporeal membrane oxygenation (ECMO) on the acute respiratory distress syndrome (ARDS) (Lewandowshi, 2000)

RELATION OF LUNG INFECTION AND CHEST PHYSICAL THERAPY

In the basic of competition between bacteria and lung defense mechanism is a change of mucus characteristic, sticky mucus secreted by the cells of pulmonary bronchi. There are substances which kill bacteria or inhibit their growth and also a mechanical trap. If mucus secretion is increased because of the changes of cells producing, lung infection may occur frequently and the deficiency of coughing out (Spector, et al., 1999B).

The control infection of inpatients who suffered with recurrent lung infection can perform in many issues such as manipulated with antibiotic, or nutrition, health care with sterile techniques. Important points that recurrent infections often occur are from atelectasis and secretion accumulation. Practical treatment with atelectasis and secretion obstruction are performed by chest physical therapist (CPT) in order to resolving, prevention, and then promotion the lung function (Decasare, et al., 1995; Sadowshy, et al., 1994).

Techniques of chest physical therapy are composed of postural drainage, percussion, vibration, and suction (Eubanks, et al., 1985; Levitzky, et al., 1990). The equipment used in the treatment are a ventilator, a vibrator, an ultrasonic neubulizer, humidifier, and a oxygen tank.

Postural drainage is a positioning of the patient's body for remove secretion from peripheral to central bronchus airway with gravity reinforcement. The postural drainage that is a specific position and depends on the lung lobes. (Table 3).

Table 3. The Postural drainage position on the lung lobes

Lung lobes	**Right lung**	**Left lung**
Upper lobe	upright position about 45 degree	upright position about 45 degree
Middle lobe	Supine position or neutral lying	Supine position or neutral lying
Lower lobe	Head down position about 45 degree	Head down position about 45 degree

Time treatment for each lobes or segments is about 5 to 30 minute depending on the endure of patients and severity of diseases.

Percussion and vibration are applied vibrated mechanism from hands through the chest wall. Main goal of percussion is to produce the vibrate force from the chest wall pass through the lung tissue deeply and pushing the secretion off the peripheral a airway to central airway. Various frequency of percussion less than vibration and gently force on the chest wall. Vibration is a more frequency and performance during patient exhalation. Mechanic of vibration are help to move secretion with air while an exhale in the airway. Contraindication of using both techniques are multiple rib fractures, unknown causes of hemoptysis, and subcutaneous emphysema. Suction with small-size catheters and vacuum suctor can remove secretion in the tracheobroncheal airway. In pediatric airway, pressure for suction should be lower

than 100 cmH_2O. Contraindication of suction is only progressive blood bleeding and tracheobroncheal trauma.

Standard method for chest physical therapy including timing, frequency, and protocol are depended on the condition of patients. From the guide to physical therapy practice of the American Physical Therapy Association (APTA) in 2001 provides the expected 16-84 times of visits per episode of care in the neonatal patients who classified in pattern G; impaired ventilation, respiration, gas exchange, and aerobic capacity/ endurance associated with respiratory failure in the neonate (Rothstein, 2001).

Humidity and aerosol therapy is a part of treatment for lubricanting the secretion and promoting secretion evacuation from the tracheal tree (Spearman, et al., 1982). Aerosol with humidifier is an equipment for produce the micromolecular of water and higher temperature with various techniques as pass-over, bubble-diffusion, jet, heated humidifiers. Humidification of inspired gas has been advocated in patients with thick and tenacious secretion. Although, no studies support the role of external humidifiers in improving the character and mobilization of thick secretions. In addition to humidification, aerosal therapy with bland solution such as distill water and hypertonic saline are used to stimulate cough.

Metered dose inhalers (MDI) are freon-powered neubulizers which provide multidose convenience through use of a metering device. MDI are the most widely used form of aerosal device for administration of bronchodilators, anticholinergics and steroids drugs. A number of many styles of adaptors that use to optimize drug delivery in the intubated patients are aviable. (Peters, et al., 1982). But regular treatment in the neonatal patients with endotracheal tube is performed with metered dose inhaler (MDI). The technique for use of an MDI with the ventilator can be performed on hand with ambu bag. The duration time is about 10 minute per time. (Holt, 1994)

BIOMARKERS IN THE FREE RADICAL AND ANTIOXIDANTS

Previous data has shown the various markers of free radical activity and antioxidant capacity. The possible markers that seems related with lung infection are lipid peroxidation product (.i.e. MDA), and glycosaminoglycan oxidation product (i.e. HA), whereas the antioxidants are GSH and Vit E. Most of studies in the lung infection have shown that free radicals products are inversely correlation with the antioxidants determined in whole blood, plasma or serum (Woodford, et al., 1998).

Airway secretion is one of interesting samples for the clinical evaluation of lung defense from infection. In normal healthy secretion is composed of high molecular-weight macromolecules (also called mucins) linked with proteins and lipids to form a gel network. Different biochemical components are involved in the gel like of properties of airway secretion. Protein, glycoproteins, proteoglycans and lipids are bound to ions and water. The functional properties of airway secretion are mainly two parts, transport of antibacterial, antioxidants and antiprotease. Another parts are filtrated and diffused substrate after infection, secretion components changed. Mucus hypersecretion is accompanied by an increase in serum protein transudate with a high content of glycoprotein, protein, proteoglycan and lipid. The biochemical abnormalities cause a marked hyperviscosity, associated with an increase in the adhesiveness and a lowering of wettability. (Puchelle, et al., 1995)

Defense mechanism of free radical in the lungs is found on the antioxidants such as GSH, Vit E concentrations in the blood. But local defense in the lung is very interesting. New studies have been performed in the lung tissue and bronchoalveolar lavage (BAL) or in alveolar lining fluid (ALF). As a report that review of the free radicals are a basis of air pollution by focusing on ozone. GSH concentration in the alveolar lining fluid or BAL is higher than in plasma (30-300 μM, and 2-6 μM respectively), whereas Vit E concentration in BAL is less than in plasma (4 μM and 10-40 μM respectively). (Kelly, et al., 1995. (Figure 13)

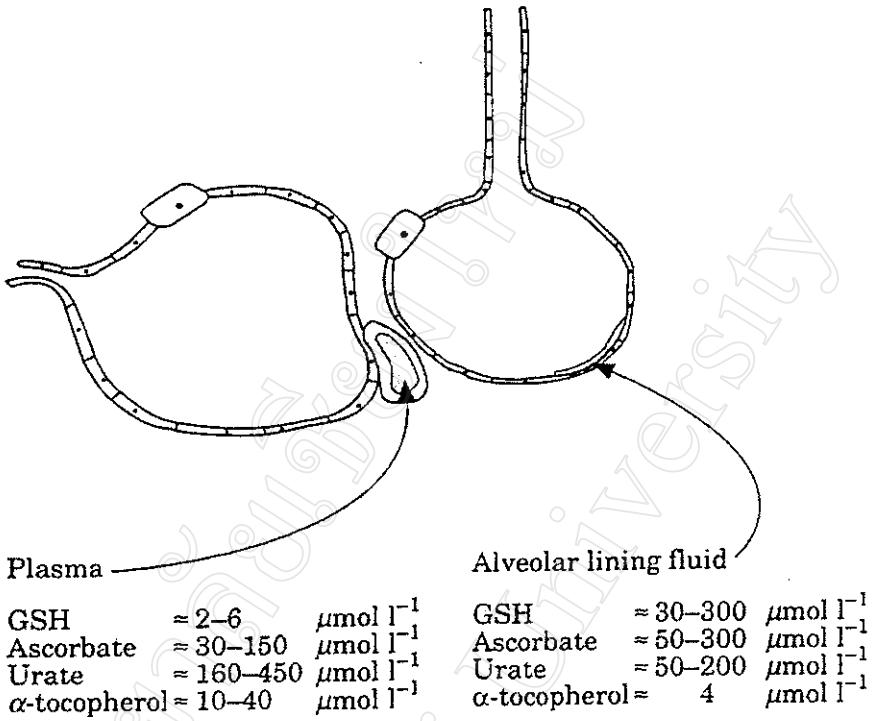


Figure 13. The levels of antioxidants in plasma and lung epithelial lining fluid. (Kelly, et al., 1995)

Tracheal aspirate (TA) from routine suction in the tubes is very interesting because it can be easily obtained by non-invasive method. All patients are on incubated with on ventilator, so tracheal aspirate is suitable. For clinical studies of free radical activity in the patients with lung infection, determining MDA in TA is suitable and useful like as BAL (Nowek, et al., 1996; Petruska, et al., 1990).

TA and sputum are still used to determine the antioxidant substances such as GSH and Vit E. Some evidences involving TA or sputum in idiopathic pulmonary fibrosis patients show the low GSH concentration in the epithelial lung fluid (ELF) (Danletbaev, et al., 2001). Determination of GSH concentration in sputum in asthma patients and healthy subjects are found that total glutathione concentration in healthy

subjects show only 3.9 μmol/L (1.0-12.3 μmol/L), while as in asthma patients show 6.4 μmol/L (1.3-19.2 μmol/L) (Boda, et al., 1998). Some data determination the GSH concentration to relative with phospholipid concentration in the lung GSH/PL ratio such as, in TA of 32 incubated premature and newborn infants in neonatal intensive care unit (NICU) with IRDS found only 2 nM/μM, whereas in healthy subjects found 127 nM/ μM (Karp, et al., 1986).

Vitamin E is also present in extracellular fluid, where it is carried with circulating lipids. The level of plasma Vit E is from 5 to 20 μM in healthy persons, less than 5 μM in Vit E-deficient patients and only 21 ng/ml in BAL fluid (Shock, et al., 2001). In children with mean ages 7.2 year, their Vit E in BAL found 0.0028-0.0073 μM (Smith, 1976). Therefore, tracheal aspirates (TA) is a very interesting samples for evaluating the oxidative stress and antioxidant from lung infection.

HYPOTHESIS OF THIS STUDY

Previous data have shown relationship between free radical and antioxidant defense in the lung, i.e. MDA, HA, GSH, and Vit E. Free radicals are produced in lung infected with bacteria or virus by the activity of macrophage or neutrophils in the normally inflammatory process. Sputum accumulation in the airway induces to lung atelectasis and recurrent infection, that stimulates the free radicals activity. Increasing knowledge in this study whether removing the secretion by chest physical therapy can change the oxidative stress in the lung by determining malondialdehyde (MDA) from lipid peroxidation, hyaruronic acid (HA) from proteoglycan degradation, glutathione (GSH), and alpha-tocopherol (Vit E).

The concentrations of all substances were determined in the blood and tracheal aspirates in which contain lots of microbes for recurrent infection. These substances may be correlated with severity of the lung injury and it might be clinical markers for follow up the lung improvement. The hypothesis illustration diagram is shown in the Figure14.

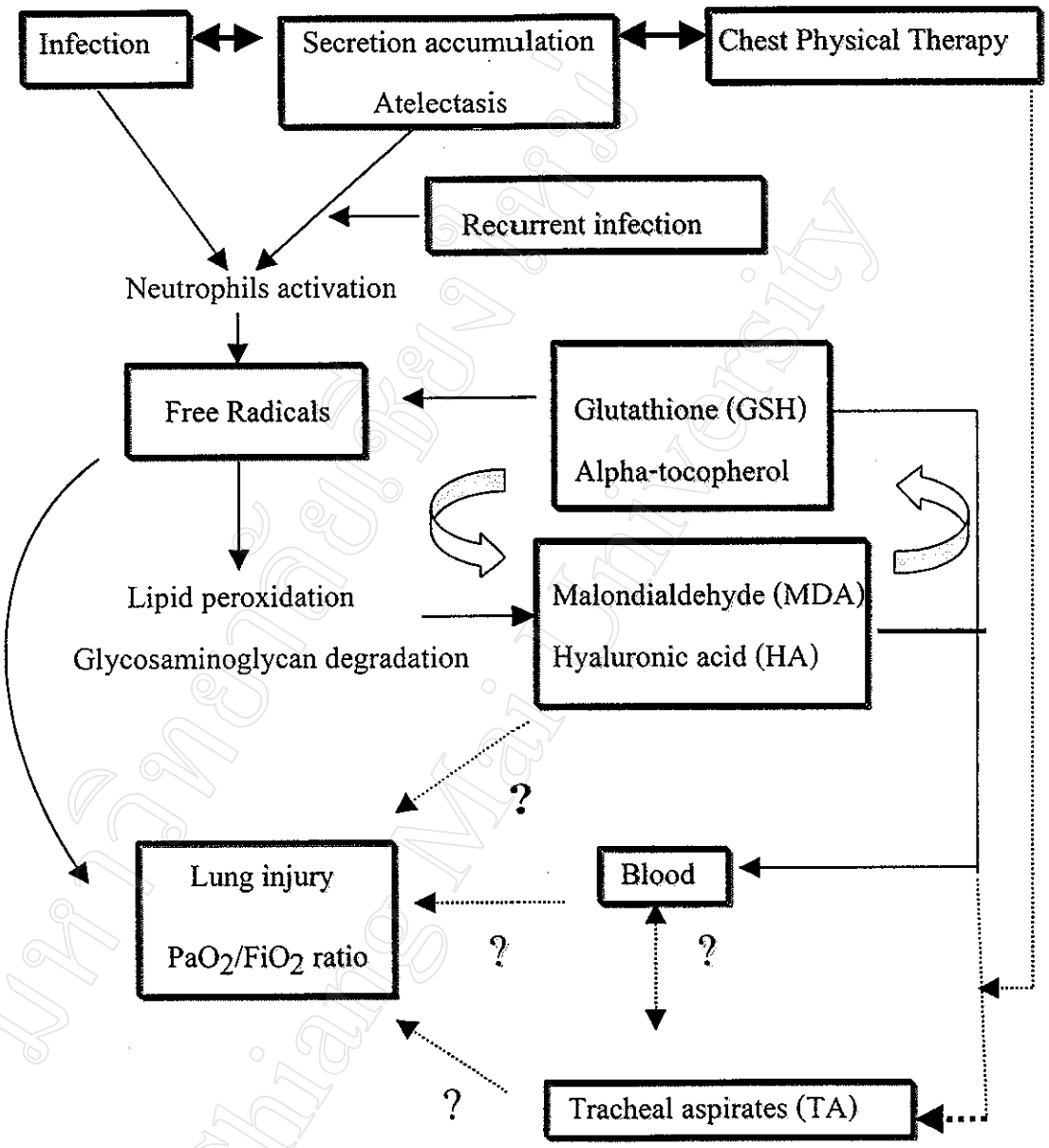


Figure 14. The hypothesis illustration of chest physical therapy (CPT) for preventing secretion accumulation or atelectasis that attach microbia. Whether CPT shows the effects on the biomarkers .i.e., MDA, HA, GSH, alpha-tocopherol, or lung injury severity in pediatric patients after lung infection.

OBJECTIVES

1. To determine the changes of malondialdehyde (MDA), reduced glutathione (GSH), alpha-tocopherol (Vit E), and hyaluronic acid (HA) in the blood and tracheal aspirate (TA), lung injury score, and oxygenation index (PaO_2/FiO_2) on the first and sixth day of two difference physical therapy's techniques in pediatric patients after lung infection.

2. To compare the malondialdehyde (MDA), reduced glutathione (GSH), alpha-tocopherol (Vit E), and hyaluronic acid (HA) in the blood and in the tracheal aspirate (TA) samples.

3. To determine the relationship between malondialdehyde (MDA), reduced glutathione (GSH), alpha-tocopherol (Vit E), and hyaluronic acid (HA) in the blood and tracheal aspirate (TA) with severity of the lung injury.