Chapter 5
Pulmonary Infections—Oxidant Injury and Role of Antioxidants

Bidyalaxmi Devi Leishangthem, Ruchi Rastogi, and Archana Bhatnagar

Infections of the respiratory system caused by infecting microorganisms like bacteria, viruses, fungi, and protozoa may occur through three persuasive routes: tracheobronchial tree, pulmonary vasculature, and via direct spread from infection in the mediastinum, chest wall, or upper abdomen. Once the microbe gains entry into the tissue, the moist, natural aerobic environment of lungs provides a favorable field to flourish, making the respiratory tract susceptible to infections. The micro-organisms, however, need to overcome a large network of pulmonary defenses [1].

5.1 Pulmonary Infection

Some of the bacterial species which cause infection in the lungs are *Streptococcus pneumoniae*, *Haemophilus influenza*, *Staphylococcus aureus*, *Pseudomonas* spp., *Acinetobacter* spp., *Mycobacterium tuberculosis*, *Legionelia* spp., and others. Infection is characterized by infiltration with polymorphonuclear neutrophils and histiocytes, as well as by tissue destruction, necrosis, cavitation, and formation of lung abscesses.

Viral infections caused by influenza, parainfluenza, adenovirus, coxsackie, echovirus, varicella, vaccinia, and measles viruses sometimes lead to viral pneumonia. It is characterized by alveolar wall thickening and infiltration of lymphocytes due to secretion of proteinaceous exudative material. Rhinovirus, a small non-enveloped single-stranded RNA virus is also associated with respiratory tract infections. In case of viral lung infection, huge invasion of macrophages and neutrophils generate the reactive oxygen species (ROS) which then become important players in the disease pathogenesis. Fungal infection can be similarly caused by inhalation of

B.D. Leishangthem • R. Rastogi • A. Bhatnagar (✉)
Department of Biochemistry, Panjab University, Chandigarh 160014, India
e-mail: bidyagcg@gmail.com; ruchirastogi01@gmail.com; bhatnagar@pu.ac.in

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spores which may later cause latent infection by the conidia. Various forms of fungal infections involving the airways include histoplasmosis, coccidioidomycosis, blastomycosis, cryptococcosis, and aspergillosis. Other organisms like nocardia, candidia, yeast, protozoa, and some tapeworms may also cause respiratory infections.

5.2 Oxidant Injury and Pulmonary Infection

Oxidant injury is caused by the oxygen-derived products, free radicals like superoxide anion, hydrogen peroxide, and hydroxyl radicals which are normally produced inside the cells during cellular processes and electron transport chain. It is highly reactive and may disturb cell structure and function resulting in cell injury and death. There are many enzymes which scavenge these oxygen intermediates. Lungs constitute one of the major target organs of oxygen injury because of maximum exposure of the cells of the airways to oxygen and a large surface area of blood supply [2–4].

The ROS are produced mainly by two organelles of the cell i.e., mitochondria and endoplasmic reticulum. ROS are produced not only during molecular reduction of oxygen in electron transport chain but also by other mechanisms like the respiratory burst in phagocytes, damage of cell component by ionizing radiation, and also as byproducts of various enzymes like nitric oxide synthase (NOX), xanthine oxidase (XO), and uncoupled endothelial nitric oxide synthase (eNOS). So ROS has beneficial roles in the physiological function of cells in response to factors such as the shear-stress and immunological defense system, thereby preventing damage from foreign pathogens.

Mitochondrial ROS (mROS) are superoxide molecules derived from oxygen produced at different sites of mitochondria. The mROS on one hand can cause damage to the cell, while on the other hand, can also help in the regulation of physiological functions like adaptation to hypoxia, regulation of autophagy, immunity, differentiation, and longevity of cell. Production of mROS may serve as an alarm of the cell, suggesting a change in the extracellular environment that has been induced by stresses like hypoxia, starvation, infection, and growth factor stimulation. A correlation exists between the gravity of stressor and quantity of mROS induced, implying increased production of mROS to increased stress, leading thereby to cell damage/cell death. Because of the dual role of mROS in the cell, it may be difficult to use them as targets of therapy. The effects of antioxidants on mROS also vary with changes in environmental conditions. The molecular targets of mROS for cellular adaptation during stress in different environmental conditions need further studies [5].

5.3 Oxidative Injury by Microorganisms

Pulmonary oxidant stress is an important characteristic of acute lung injury (ALI) [6]. Reactive oxygen intermediates (ROI) produced by the cells take part in host defense against infections caused by bacteria and fungi. They could bring about

destruction of proteins, deoxyribonucleic acids, and lipids. So, it is important to calibrate the ROI for effective antimicrobial defense while averting inflammation and injury. Production of ROS like superoxide anion, and its derivatives hydrogen peroxide and peroxynitrite, have been found to be associated with lung injury caused by influenza viruses [7]. In the earlier studies, pyran polymer-conjugated superoxide dismutase (SOD) when administered to virus-infected mice brought about reduction in mortality [8]. The peroxynitrates can also cause the oxidation of antioxidants like glutathione reductase, SOD, and glutaredoxin, leading thereby to lung injury. Oxidant injury was also found to be boosted in the mitochondrial membrane with an increment of mitochondrial generation of ROS in tissues distant from the lungs [9]. This study also revealed that oxidant injury and metabolic stress contributed directly to disease development.

5.4 Mycobacteria

Tuberculosis caused by *Mycobacterium tuberculosis* (Mtb) is also found to be associated with oxidative stress. Tuberculosis involves the poor antioxidative defense, damaging the host tissue. Mtb has the ability to survive during the redox status of the host, and also the ability to use protective enzymes like SOD, catalase (KatG), alkyl hydroperoxidase (AhpC), and peroxiredoxins [10]. Mycobacteria induce the production of ROS production by mononuclear and polymorphonuclear phagocytes. Chemotherapy given to patients of pulmonary tuberculosis showed an improvement in the level of oxidative stress [11]. The production of ROS is also associated with the productions of DNA lesions using apurinic/apyrimidinic endonuclease IV (End) and exonuclease III (XthA), a 39R59 exonuclease [12]. Pulmonary and extra pulmonary tuberculosis are associated with decreased levels of blood glutathione, glutathione peroxidase, and glutathione reductase and negatively correlated with carbonyl protein content [13]. Since γ-glutamylcysteinylglycine or glutathione (GSH) protects against oxidative stress, it may have potential therapeutic implications [14]. Also, ergothioneine (ERG) and mycothiol (MSH), have been reported in protection against oxidative stress in mycobacterium [15].

Mycobacterium tuberculosis is one of the aerobic bacteria which can survive under oxidative stress by various mechanisms. It has the ability to persist inside macrophages of the host. One of the mechanisms is F420-dependent anti-oxidant mechanism. This system in methanogenic archaea acts as an active enzyme cofactor. It has been observed that the F420-deficient mutants (by the inactivation of fbIC gene) are very sensitive to oxidative stress. The *fbiC* gene (Rv1173) encodes an 856-amino-acid polypeptide FO synthase in the F420 biosynthetic pathway. The inhibition of the F420 biosynthesis pathway or Fqr-class proteins may act as a mechanism to potentiate the action of bactericidal agents [16]. To study as to how the mycobacterium is able to evade the host immune system, state-specific models, based on readily available gene expression data, can be created in silico. In such models, the metabolic adaptations of *M. tuberculosis* can be characterized by the

differential gene expression data with a metabolic network model [17]. More molecules are reported to be involved in protection of the microbes against oxidative stress as seen in mycoredoxin-1 and mycothiol deletion strains of *Mycobacterium smegmatis* [18]. The survival of pathogen i.e., *M. tuberculosis* inside the host may also be possible by oxidation-sensing regulator (MosR), a transcriptional repressor by upregulating expression of rv1050 (a putative exported oxidoreductase) [19].

Heme oxygenase-1 (HO1) has a role in cytoprotection and is found to be expressed in large numbers in the plasma of patients with tuberculosis, acute respiratory distress syndrome, chronic obstructive pulmonary disease, and asthma. The expression of HO-1 therefore has been suggested to be used in prognosis of lung disease [20]. A signal transduction pathway in the Early Secreted Antigenic Target of 6 kDa induction of IL-8 expression in lung epithelial cells which has been identified might be important to understand the innate immune responses to tuberculosis and the pathogenesis of lung injury in tuberculosis [21].

There are reports on the role of serine proteases of the Mtb that provide resistance to acid and oxidative stress. Rv3671c, a putative serine protease is held responsible for persistence of *Mycobacterium tuberculosis* in the hostile environment of the phagosome [22]. The periplasmic domain of Rv3671c is a functional serine protease of the chymotrypsin family and its activity was found to increase upon oxidation. On similar lines, another periplasmic protease of Mtb might have a special role in imparting resistance to acid and oxidative stress [23]. This transmembrane serine protease MarP is important for pH homeostasis in Mtb.

5.5 Bacteria

Bacterial infection leads to the exposure of bacteria-derived lipopolysaccharides, composed of oligo, polysaccharide, and lipid A endotoxin to the host tissue. Lungs are very sensitive to endotoxins; acute endotoxemia directs accumulation of macrophages in the target tissues. The macrophages and neutrophils that reach the infected site are activated by LPS releasing reactive oxygen and nitrogen species contributing to injury and organ failure [24].

Pseudomonas aeruginosa causes acute and chronic infections of the human lung, causing tissue injury. A siderophore (iron bound to pyochelin) secreted by the organism to acquire iron, may actually function as an efficient catalyst for hydroxyl radical ($HO^{\bullet}$) formation. Due to exposure of superoxide ($O_2^{\bullet -}$) and H_2O_2, siderophore can augment injury to pulmonary artery endothelial cells [25]. Therefore, the presence of ferripyochelin at sites of lung infection by *P. aeruginosa* might promote $HO^{\bullet}$ mediated damage to airway epithelial cells resulting in tissue injury.

Lung infection caused by *Bordetella pertussis* shows a stimulated innate resistance (StIR) event which is also mediated by the generation of ROS [26].

There is evidence of inflammation and oxidative injury in bronchopulmonary dysplasia, a neonatal chronic lung disease. The inflammation associated with dysplasia is caused by the exposure of bacterial lipopolysaccharides. Bacterial LPS

bring about increased expression of cytokines regulated by Nox-dependent signaling pathways [27, 28]. The inflammatory mediators help in attracting neutrophils and macrophages to the lungs to combat infection. Neutrophils, after stimulation produce ROS like hypochlorous acid (HOCl) by the help of myeloperoxidase enzyme. The levels of glutathione sulfonamide (GSA) (fourfold) and other neutrophil oxidant biomarkers (twofold) were reported to be significantly higher in culture positive aspirates. GSA is a stable oxidation product of GSH that is formed by condensation of the amine group of the γ-glutamyl residue with the oxidized cysteine. This has led to the recommendation of GSA as a marker of detection of bacterial growth in lung infection [29].

Nontypeable *Haemophilus influenza* (NTHI) is a major cause of acute sino-pulmonary infections, responsible for exacerbations of COPD. During *H. influenza* infection, many stimuli that include reactive oxidants, bring about induction of Nuclear erythroid factor-2 (Nrf2), a basic leucine transcription factor. It detaches from its inhibitor Keap1 present in the cytosol and moves toward nucleus where it binds at the promoter region of antioxidant response elements (AREs) and help in protection against oxidant injury [30]. Also, during infection, *H. influenza* can withstand the effect of ROS produced by the cells, has various molecular mechanisms to protect from the stress [31].

5.6 Oxidant Injury by Viruses

The involvement of oxidative stress during viral infection has been also reported [32]. The addition of environmental contaminant like cadmium induced oxidative stress leading to imbalance in the redox state with reduction in GSH. However addition of antioxidants like GSH derivative (GSH-C4) or the GSH precursor, *N*-acetyl-L-cysteine (NAC) result in the inhibition of viral replication as studied in Madin Darby Canine Kidney [33].

Human immune-deficiency virus (HIV) patients are under constant oxidative stress as reflected in alterations in levels of ascorbic acid, tocopherols, carotenoids, selenium, SOD, and glutathione in various tissues. Elevated levels of hydroperoxides and malondialdehyde in serum are also indicative of oxidative stress during HIV infection. The oxidative stress contributes to HIV disease pathogenesis, viral replication, inflammatory response, reduced immune cell proliferation, loss of immune function, apoptosis, chronic weight loss, and increased sensitivity to drug toxicities [34]. A chronic infection with HIV is known to be associated with an incidence of pulmonary complications including hypertension, vasculopathy, lymphocytic alveolitis, and interstitial pneumonitis, not attributed to either opportunistic infections or presence of the virus. A transactivator, Tat (transactivator of transcription) protein is required for expression of full-length of viral genes, and it influences the expression of cellular inflammatory genes. The Tat-dependent transactivation of genesis known to require specific mediators that include the transcription factor, NF-κB, are known to be sensitive to changes in cellular oxidant burden [35].

The HIV-1 transgenic mice demonstrate significant oxidative/nitrosative stress in the lungs upon administration of endotoxin. This suggests that the pulmonary complications in HIV-1 infections could be due to alteration of the lung proteins by oxidative stress [36]. HIV-related proteins and alcohol together cause dysfunction in the lung epithelium. This is a significant observation as the alveolar barrier gets affected and addition of thiol antioxidant results in improvement in transgenic mice [37]. Human immunodeficiency virus 1-infected individuals display systemic oxidative stress and glutathione deficiency. The master transcription factor nuclear factor (erythroid-derived 2)-like 2 is known to regulate the expression of antioxidant and phase II-metabolizing enzymes by activating the ARE that protects cells and tissues from oxidative stress [38].

Respiratory syncytial virus (RSV) infects the lower respiratory tract in children. It has been found to generate ROS in vitro and oxidative injury in lungs in vivo [39]. Oxidative stress induced by RSV is due to inhibition of antioxidant enzyme expression leading to an imbalance of ROS production and airway antioxidant defenses [39]. There is an increment in lipid peroxidation products and decrement in GSH/GSSG ratio in RSV infected cells [40]. RSV is also one of the primary causes of lower respiratory tract infections in most parts of the world during the first year of life. Infiltrating lymphocytes present in bronchoalveolar lavage fluid (BALF) have been observed in mice infected with a lethal dose of influenza A/PR8/34 virus which demonstrated the role of oxidative stress during lung infection [41].

It has been reported that resveratrol treatment reduced the number of infiltrating lymphocytes and RSV lung titers which finally led to reduced inflammation. Furthermore, resveratrol might help to decrease the IFN-γ levels in BALF of RSV-infected mice by attenuating airway responses to methacholine. Resveratrol inhibited the TIR-domain-containing adapter-inducing interferon-β (TRIF) signaling pathway, controlled Toll-like receptor 3 (TLR3) expressions and also induced M2 receptor expression followed by RSV infection [42]. A significant fall in SOD 1, SOD 3, catalase, and GST expression was found but SOD 2 expression was found to be elevated [40]. It was also observed that there was a decrement in the activity of SOD, catalase, glutathione S-transferase, and glutathione peroxidase in murine lungs and in the airways of children with severe bronchiolitis. It was associated with reduced levels of Nrf2 expression in the lungs of viral infected mice [43].

The role of antioxidants in the RSV infection has been analyzed from the effect of butylated hydroxyanisole (BHA), an antioxidant, in the RSV infected BALB/c mice; a fall in malondialdehyde and 4-hydroxynonenal content in bronchoalveolar lavage of infected mice indicated the reduction in lung oxidative stress. BHA treatment caused a drop in clinical illness and body weight loss with the neutrophil recruitment to the lung and pulmonary cytokine and chemokine production after the infection. Along with these findings, there was a reduction in RSV-induced airway hyper reactivity [44].

Increased production of superoxide, increased activity of xanthine oxidase, oxidized glutathione, malondialdehyde, and decreased production of oxidized glutathione are some of the characteristics associated with respiratory viral infections [33].

Rhinoviruses infection is associated with an increase in levels of intercellular adhesion molecule (ICAM-1), an important molecule which is used as receptor for entry in the pulmonary epithelial cells. Increased levels of superoxide formation are associated with increased ICAM-1 expression in rhinovirus infection [45]. An increased involvement of oxidative stress with adenovirus-induced lung infection is reported as the cause of bronchiolitis obliterans in post-transplant patients. The association of post infectious bronchiolitis obliterans with oxidative status in the lungs of children has also been reported [46].

5.7 Sepsis and Pulmonary Infection

Sepsis results from serious infections caused by various pathogenic organisms like bacteria, viruses, and fungi which lead to multiple organ failure. Oxidative stress has been shown to be associated with sepsis. In a comparative study on the effects of oleanolic acid with dexamethasone on inflammation and apoptosis in lung and distal organs in experimental murine sepsis, oleanolic acid was associated with lower induced nitric oxide synthase (iNOS) expression and higher SOD levels than in the dexamethasone treated group [47]. There are reports on the improved renal and pulmonary function in rats with sepsis, treated with potent antioxidant NAC [48]. ROS elevate vascular barrier dysfunction through Ca^{2+} signaling in the sepsis-induced ALI [49]. Previous studies had also revealed that pulmonary oxidative stress generated in murine sepsis-induced ALI was primarily dependent upon neutrophil iNOS among different isoforms of NOS [50–52]. Similarly in severe viral infections, the level of inducible nitric oxide was found to be higher in mice that were infected with H5N1 and 1918 viruses, in comparison to a seasonal H1N1 virus in lung tissue; the level was moderate in mice that were deficient in iNOS ($NOS2^{-/-}$) in comparison to wild-type control [51]. Additionally, this study also showed the delay in weight loss and death in 1918 virus-infected mice in contrast to control ones when treated with NOS inhibitor, NG-monomethyl-L-arginine [53].

5.8 Antioxidants in the Therapy of Infections

Antioxidants are substances that inhibit the oxidation of other molecules by terminating the chain reaction of free radicals and removing their intermediates via self oxidation [2]. There are several types of antioxidants such as glutathione, vitamin A, vitamin C, vitamin E, as well as enzymes like catalase, SOD, and various peroxidases. Antioxidant enzymes play an important role in defense against oxidative stress in the lung and in the pathogenesis of chronic respiratory diseases. Extracellular SOD, an important antioxidant enzyme, is found in the lungs, it controls pulmonary inflammation and injury by promoting bacterial phagocytosis [54].

The role of Nrf2-mediated antioxidant system to defend the lungs from oxidative injury and inflammation has also been shown in in vitro and in vivo studies. The in vitro study reports augmentation of NF-κB activation and induction of its target inflammatory gene in Nrf2-deficient macrophages vs. the wild type, when these macrophages were examined with poly (I:C) and/or cigarette smoke extract. There was also an enhancement in antioxidant genes in the lungs of wild-type mice as compared to Nrf2-deficient mice after cigarette smoke exposure in the in vivo study [54]. Mortality was found to be higher in cigarette smoke-exposed Nrf2-deficient mice when these mice were infected with influenza virus [55]. Neu-164 and Neu-107, inhibitors of myeloperoxidase enzyme, exhibiting strong antioxidant activity, were found to reduce acute inflammation and oxidative stress triggered by cigarette smoke-induced inflammatory cells through scavenging the ROS [56]. The drugs ketamine, propofol, and ketofol are routinely used for sedation, but a recent report highlights their role on oxidative stress and anti-inflammatory processes in lung tissue in a rodent model of endotoxemia [57]. In the sepsis (LPS mediated) induced ALI, ketamine infusion led to reduction in the levels of TNF-α, IL-1β, IL-6, NF-κB, and COX-2 mRNAs in lung tissue. Propofol was involved in lessening the levels of circulating TNF-α and IL-1β in lung tissue, whereas it led to augmented nitrate/nitrite levels. The third drug, Ketofol, reduced the levels of COX-2 mRNA and the nitrate/nitrite level in lung tissue. However, a recent report suggests that the treatment with antioxidants in excess might be deleterious as it may result in greater oxidative stress [58].

5.9 Anti-Oxidants and HIV Infection

HIV-1-related proteins inhibit Nrf2-mediated antioxidant defenses and thereby disrupt the normally tight alveolar epithelial barrier. Nrf2-RNA silencing dampened the activity of Nrf2/ARE, decreased the expression of the tight junction proteins zonula occludens-1, occludin, and claudin-18, increased paracellular permeability of alveolar epithelial monolayers derived from wild-type rats, and therefore reproduced the effects of HIV-1 transgene expression on the epithelial barrier. In contrast, upregulating Nrf2 activity, either by plasmid-mediated overexpression or treatment with the Nrf2 activator sulforaphane, increased the expression of ARE-dependent antioxidants, including NAD(P)H dehydrogenase, quinone 1, and glutathione, improved the expression of tight junction proteins, and restored the ability to form tight barriers in alveolar epithelial cells from HIV-1 transgenic rats. Taken together, these new findings argue that HIV-1-related proteins downregulate Nrf2 expression and/or activity within the alveolar epithelium, which in turn impairs antioxidant defenses and barrier function, thereby rendering the lung susceptible to oxidative stress and injury. Furthermore, this study suggests that activating the Nrf2/ARE pathway with the dietary supplement sulforaphane could augment antioxidant defenses and lung health in HIV-1-infected individuals [38].

5.10 Respiratory Viral Infections

Modulation of ROS production and oxidative stress contributes one of the therapeutic approaches in virus-induced lung infections. Use of small molecules like thiols, polyphenols, and antioxidant mimetics show effects on viral-induced ROS production and oxidative stress. Also, compounds like triterpenoids, sulforaphane, and isothiocyanates, increase endogenous antioxidant enzyme levels in RSV infection by stimulating Nrf2-dependent gene expression [39]. There may also be suppression of excessive superoxide production from NADPH oxidase 2 (Nox2), which is the primary enzymatic source of superoxide in mammalian inflammatory cells, because it markedly alleviates lung injury and virus replication caused by influenza A virus. So Nox2 oxidase inhibitors could be useful for suppression of virus-induced lung disease [8]. Mice lacking a functional phagocyte NADPH oxidase (Cybb tm1 mice) or treated with the metalloporphyrin antioxidant manganese (III) tetrakis (*N*-ethyl pyridinium-2-yl) porpyhrin (MnTE-2-PyP) show heightened inflammatory infiltrates in their airways in response to pulmonary influenza infection. Raising the resting threshold of lung-resident antigen-presenting cells by modulating homeostatic negative feedback loops may therefore provide generic protection against viral infectious disease, irrespective of the infective strain.

The treatment of lung infections caused by viruses like RSV, and bacteria like *Pseudomonas aeruginosa* may include resveratrol (3,5,4′ trihydroxystilbene) which is a natural polyphenolic compound that has antioxidant property. It helps in the attenuation of inflammatory response in bacteria infected cells. In vitro studies in A549 cells observed that resveratrol treatment significantly reduced ROS generation, human beta-defensin-2 expression, ICAM-1, increased glutathione peroxidase levels (also the markers of apoptosis), suggesting resveratrol as a protective therapeutic agent in lung infection [42, 59]. Long pentraxin 3 (PTX3) is a newly discovered acute phase protein produced at the sites of infection and inflammation by tissue cells, macrophages, monocytes, and dendritic cells. PTX3 plays an important role in preventing infection of certain fungi, bacteria, and viruses in the lung [60]. Another drug, roflumilast is also found to attenuate the RSV infection in human differentiated bronchial epithelial cells [61]. It impeded the damage of ciliated cells and lowered the escalation of MUC5AC, CLCA1, IL-13, IL-6, IL-8, TNFα, and ICAM-1. Furthermore, it also inversed the decrement of Nrf2, HO-1, and GPx mRNA levels [61].

Lung infection caused by H1N1 Influenza A is found to be associated with uncontrolled inflammation and oxidative stress. FABP5 which belongs to the family of fatty acid-binding proteins (FABPs), acts as an anti-inflammatory mediator during lung infection. FABP5 are small, highly conserved, cytoplasmic proteins that bind long-chain fatty acids and other hydrophobic ligands. They increase peroxisome proliferator-activated receptor gamma (PPAR-γ) activity resulting in reduced inflammation which shows the involvement of FABP5 in controlling the oxidative damage and inflammation during lung infection, making it a future therapeutic drug. Treatment with recombinant human thioredoxin-1 is also found to increase

the survival rate of murine model of influenza pneumonia. So thioredoxin-1 acts as an antioxidant and anti inflammatory molecule.

Interestingly, inhalation of recombinant human catalase (rhCAT) exerted protective effect by improving pathological process and by reducing the viral titer in lungs of mice exposed to influenza H1N1 viral pneumonia. Moreover, rhCAT also improved the serum ROS scavenger capacity [62]. Use of rhCAT had a limitation of its elimination from blood, hence, it was modified by coating with one of the active polymer, polyethylene glycol monomethyl ether (PEGrhCAT). The pharmacokinetics in mice revealed that it had longer half life than native rhCAT, treatment with PEGrhCAT was found to be more effective than with the native form. As the PEGrhCAT caused reduction in viral replication, lung injury levels, and ROS production, the molecule can be used as an adjuvant therapy to promote efficacy of anti viral drugs [63]. The use of hydrogen gas in saline has been used as therapeutic and prophylactic potential for the treatment of injury caused by inflammation and oxidative stress.

5.11 Natural Products as Anti-oxidants

The nutritional status plays a major role to maintain an optimal immune system. The active ingredients of the naturally derived agents may affect various domains suggesting an interdependence of optimal immune system and oxidative stress. Consumption of gold kiwi fruit leads to the reduction of plasma lipid peroxidation in those infected with upper respiratory tract infection symptoms. It may be possibly attributed to the diet-derived antioxidants which control ROS generation [64].

Pretreatment of a heteropolysaccharide, RIWP, isolated from *Radix isatidis* enhanced murine alveolar macrophage survival by inhibiting the production of ROS and lipid peroxidation after stimulation with lipopolysaccharide. Upon treatment, the murine alveolar macrophages exhibited diminished generation of nitric oxide, prostaglandin E2, tumor necrosis factor-α and IL-6, and the mitochondrial membrane potential also returned to normal conditions [65].

There are reports on the therapeutic roles of some Chinese herbal medicine for the treatment of tuberculosis. The effects of *Radix Ranunculi Ternati*, *Radix Sophorae Flavescentis*, *Prunella Vulgaris* L., and *Stellera Chamaejasme* L. extracts have been found to have the capability to enhance cell-mediated immune response in a multi-drug resistant tuberculosis model [66]. Kampo (Traditional Japanese Herbal) medicine, Hochuekkito (TJ-41), have been used since they possess a property to inhibit influenza virus replication by the regulation of interferon gamma [69]. The levels of GM-CSF and an antimicrobial peptide, defensin, are found to increase after TJ-41 treatment, so defensin might play a role in inhibiting virus replication [67].

Dioscorin, a Chinese herbal medicine possessing the effective antioxidant and anti-trypsin activities inhibited H_2O_2, a potent ROS engaged in lung and bronchial epithelium injury. The results of the study also suggested that the inhibition was

relayed by attenuation of H_2O_2 alteration on G2/M cell cycle arrest, induction of IκB, and reduction of NF-κB along with the inhibition of IL-8 secretion, and less changes in adhesion molecule expressions in H_2O_2-injured A549 cells [68].

In addition to the medicinal herbs, Wen-Pi-Tang extract also serves as a natural medicine to cure the lung injury caused by influenza virus. This virus is known to generate the xanthine oxidase (XO) activity of the lungs resulting in a higher level of oxygen-free radicals. Wen-Pi-Tang extract diminished the XO activity [69]. Another Chinese medicinal herb, *Magnolia officinalis* also possesses antioxidant activity [70]. Magnolol, the active compound of this herb, reduced the lipid peroxidation intensity in plasma, liver, and lung of rats with sepsis [70]. A Chinese herbal formula, Qing-Fei-Tang, was found to attenuate the oxygen-free radicals that were generated after stimulation of healthy human leukocytes with opsonized zymosan. It also inhibited the release of slow reacting substance of anaphylaxis from guinea pig lung when challenged with antigen. In addition, this herbal remedy re-established the loss of saturated fatty acids in sputum and showed an improvement in lung inflammation [70].

5.12 Conclusions

Different pathogens which infect the lungs cause pulmonary inflammation and oxidative stress. The activated neutrophils, macrophages, and eosinophils induce the production of singlet oxygen and hydrogen peroxide, which damage the surrounding tissues and enhance production of ROS. In the presence of different infections, the host uses a variety of antioxidants that signal through different pathways, bring about enhanced transcription factor generation leading to control of the infection/inflammation/injury.

The challenge for future research lies to establish the antioxidants which can be used in the most efficient manner for lung infection-associated oxidative stress, and which of the wide range of current oxidative stress markers can we employ? There is an intensive ongoing search for markers which would identify the patients who are most likely to encounter adverse outcomes from various lung infections. There exists an enormous potential for not only the synthetic drugs, but also of natural compounds with reduced side effects as antioxidants.

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