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OXIDATIVE STRESS IN ASTHMA: PART OF THE PUZZLE

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Abstract

An imbalance between the production of reactive oxygen species and the capacity of antioxidant defense mechanisms favoring oxidants is called oxidative stress and is implicated in asthmatic inflammation and severity. Major reactive oxygen species that are formed endogenously include hydrogen peroxide, superoxide anion, hydroxyl radical and hypohalite radical; and the major antioxidants that fight against the endogenous and environmental oxidants are superoxide dismutase, catalase and glutathione. Despite the well known presence of oxidative stress in asthma, studies that target oxidative burden using a variety of nutritional, pharmacological and environmental approaches have generally been disappointing. In this review, we summarize the current knowledge on oxidative stress and antioxidant imbalance in asthma. In addition, we focus on possible biomarkers of oxidative stress in asthma and on current and future treatment strategies using the modulation of oxidative stress to treat asthma patients.

Keywords: Asthma, antioxidant, bromotyrosine, catalase, glutathione peroxidase, nitric oxide, oxidative stress, superoxide dismutase

ABBREVIATIONS

AP-1: Activation protein-1

C-ACT: Childhood asthma control test

COPD: Chronic obstructive pulmonary disease

EBC: Exhaled breath condensate

EC-SOD: Extracellular superoxide dismutase

EPO: Eosinophil peroxidase

ETC: Electron transport chain

FeNO : Fractional exhaled nitric oxide

FEV1: Forced expiratory volume in 1 second

GSH: Glutathione

GSH-Peroxidase (GSH-Px): Glutathione Peroxidase

HIF-1: Hypoxia inducible factor-1

H₂O₂ : Hydrogen peroxide

HOCl: Hypochlorous acid

LDH: Lactate dehydrogenase

O₂⁻ : Superoxide anion

•OH : Hydroxyl radical

MAPK: Mitogen activated protein kinase

MPO: Myeloperoxidase

NAC: N-acetyl cysteine

NAD(P)H oxidase : Nicotine adenine dinucleotide phosphate oxidase

NF- κ B: nuclear factor- κ B

RNS: Reactive nitrogen species

Nrf2: Nuclear erythroid 2 p45-related factor 2

ROS : Reactive oxygen species

SODs: Superoxide dismutases

Sp1: Transcription factor Sp1, also known as specificity protein 1.

Trx: Thioredoxin

1. INTRODUCTION: What is Oxidative Stress?

Oxidative stress is defined as an imbalance between reactive oxygen species and the ability of the biological system to detoxify the reactive intermediates or to repair the damage caused by oxidative radicals¹. Oxidants, also called reactive oxygen species (ROS), are produced by living organisms from molecular oxygen as a result of normal cellular metabolism. Besides the endogenous oxidants, exogenous oxidants such as air pollutants, cigarette smoke, heavy metal ions and ionizing radiation contribute to the redox (regulation of reducing and oxidizing) status of cell. Redox state is critical for function of cells and organs. Reactive oxygen species are highly reactive molecules. Normally, they function in physiologic cell processes, but at high concentrations they can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions.²⁻⁴. Antioxidants are usually effective in blocking harmful effects of ROS. However, under pathologic conditions the antioxidant systems can be overwhelmed and oxidant stress and injury ensue.

a) Oxidants:

Reactive oxygen species can be produced endogenously by the living organism as well as by exogenous sources (Table 1).

Endogenous Sources of ROS and their actions

ROS are produced as a result of normal cellular metabolism within the mitochondria, cytochrome P450 system, peroxisomes, and inflammatory cells.⁵⁻⁷ Physiologically important ROS molecules that are produced by intracellular reduction of molecular oxygen are superoxide anion (O_2^-), hydroxyl radical ($\bullet OH$), and hydrogen peroxide (H_2O_2).

O_2^- is mostly produced in mitochondria as a result of leakage of electrons from the electron transport chain system. Other systems that mediate O_2^- production are the nicotinic adenine dinucleotide phosphate [NAD(P)H] oxidase that is found in phagocytes and the xanthine oxidase systems. O_2^- is converted into H_2O_2 and molecular oxygen by the action of superoxide dismutases (SODs).⁸ H_2O_2 is also produced by xanthine oxidase, amino acid oxidase and NAD(P)H oxidase by consumption of molecular oxygen in metabolic reactions.^{9,10}

$\bullet OH$ is the most reactive of ROS produced by breakdown of H_2O_2 in Haber Weiss and Fenton reactions in the presence of transition metals like Fe^{2+} or Cu^+ .¹¹ O_2^- itself can also react with H_2O_2 and generate OH^- .^{12,13} $\bullet OH$ can damage proteins, lipids and carbohydrates and DNA. It can also start lipid peroxidation by taking an electron from polyunsaturated fatty acids.

H_2O_2 is converted to hypochlorous acid (HOCl). HOCl is highly oxidative and plays an important role in killing of the pathogens in the airways. HOCl can also react with DNA and induce DNA protein interactions.^{11,14} In relevance to inflammatory diseases such as asthma, granulocytic enzymes further expand the reactivity of H_2O_2 via eosinophil peroxidase (EPO) and myeloperoxidase (MPO). EPO and MPO contribute to the oxidative stress by modification of proteins by halogenation, nitration and protein cross links via tyrosyl radicals.^{15,16}

Exogenous Sources of Oxidants and their actions

Exogenous ROS can be produced as a result of smoking, ozone, hyperoxia, heavy metals, UV and ionizing radiation.

Cigarette smoke contains many oxidants and free radicals and organic compounds such as superoxide and nitric oxide.¹⁷ Increased production of reactive oxygen and nitrogen species have been observed in hyperoxia, as well.¹⁸

Ozone exposure can result in lipid peroxidation, influx of neutrophils into the airway epithelium and release of inflammatory mediators such as MPO, ECP and LDH.¹⁹ In the presence of O₂, ionizing radiation converts hydroxyl radical, superoxide and organic radicals to hydrogen peroxide and organic hydroperoxides. These hydroperoxide species react with redox active metal ions such as Fe and Cu via Fenton reactions and thus induce oxidative stress.²⁰

Heavy metal ions such as iron, copper, cadmium, mercury, nickel, lead and arsenic can induce generation of reactive radicals and cause cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA.²¹

b) Antioxidants

Antioxidants can be divided into two categories: enzymatic and non-enzymatic (Table 2).

Enzymatic Antioxidants

The major enzymatic antioxidants of the lungs are superoxide dismutases, catalase and glutathione peroxidase (Figure 1). In addition to these major enzymes, other anti-oxidants including heme oxygenase-1, redox proteins such as thioredoxins, peroxiredoxins and glutaredoxins have also been found to play crucial roles in the pulmonary antioxidant defenses.

Since superoxide is the primary ROS produced from a variety of sources, its dismutation by SOD is of primary importance for each cell. All three forms of SOD, i.e., CuZn-SOD, Mn-SOD and EC-SOD (Extracellular SOD) are widely expressed in the human lung.⁸ Mn-SOD is localized to the mitochondria matrix and EC-SOD is primarily localized in the extracellular matrix. H₂O₂ that is produced by the action of SODs or the action of oxidases such as xanthine oxidase is reduced to water by catalase and the GSH-Peroxidase (GSH-Px). Catalase also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme.²²

GSH-Pxs are the enzymes responsible for the reduction of H₂O₂ as well as lipid hydroperoxides.^{23,24} The GSH-Pxs, contain selenocysteine within the active site and use low-molecular-weight thiols such as GSH to reduce H₂O₂ and lipid peroxides to their corresponding alcohols.

Non-enzymatic antioxidants

These include low molecular weight compounds such as vitamins (vitamins C and E), beta carotene, glutathione, melatonin, curcumin and polyphenols. These compounds have attracted great attention as possible anti-oxidant agents.

Vitamin C (Ascorbic acid)

Water-soluble vitamin C provides intracellular and extracellular aqueous-phase antioxidant capacity primarily by scavenging oxygen free radicals. Its plasma levels have been shown to decrease with age.²⁵ Among the best sources are orange, sweet red peppers and tomato.²⁶

Vitamin E (Alpha tocopherol)

Lipid-soluble vitamin E is concentrated in the hydrophobic interior site of cell membrane and donates electron to peroxyl radical which is produced during lipid peroxidation. α tocopherol is the most active form of vitamin E and the major membrane bound antioxidant in the cell. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formation.²⁷ Vegetable oils (sunflower and olive), non-citrus fruits, and nuts and seeds are important sources in the diet.²⁶

Glutathione

Glutathione is highly abundant in all cell compartments and is the major soluble antioxidant. GSH/GSSG ratio is a major determinant of oxidative stress. GSH serves as an antioxidant in a multitude of ways.²⁸ It detoxifies hydrogen peroxide and lipid peroxides via action of GSH-Px. Glutathione (GSH) donates its electron to H_2O_2 to reduce it into H_2O and O_2 . Oxidized glutathione (GSSG) is reduced into GSH by glutathione reductase which uses NAD(P)H as the electron donor. Glutathione peroxidases are also important for the protection of cell membrane from lipid peroxidation. Reduced glutathione donates protons to membrane lipids and protects them from oxidant attacks.²⁹

GSH is a co-factor for several detoxifying enzymes such as GSH-Px and transferase. It has a role in converting vitamin C and E back to their active forms. GSH protects cells against

apoptosis by interacting with pro and anti-apoptotic signaling pathways.²⁸ It also regulates and activates several transcription factors, such as AP-1, NF- κ B and Sp1.

Carotenoids (β -carotene)

Carotenoids are pigments found in plants. Primarily, β -carotene has been found to react with peroxy ($\text{ROO}\cdot$), hydroxyl ($\cdot\text{OH}$), and superoxide radicals ($\text{O}_2^{\cdot-}$).²⁹ Both carotenoids and retinoic acids are capable of regulating transcription factors.³⁰ Carotenoids also affect apoptosis of cells. Antiproliferative effects of retinoic acid (RA) have been shown in several studies.³¹ Best sources of β -carotene in diet are potato, liver, spinach, fish and carrot.²⁶

Melatonin

Melatonin is a hormone synthesized from serotonin primarily in the mammalian pineal gland. It can be found in dietary sources such as oats, yeast, and other plants. It is effective in neutralizing $\text{HO}\cdot$ and peroxy radicals, $\text{CO}_3^{\cdot-}$, NO_2 , $\text{O}_2^{\cdot-}$, and HOCl .³²

Polyphenols

Polyphenols are group of antioxidants and consist of flavonoids, phenols, phenolic acids, lignins, and tannins. found in the fruits, vegetables, nuts, red wine , beer, tea, seeds, grains, spices, and medicinal plants The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals. Flavonoids inhibit xanthine oxidase, cyclooxygenase, lipooxygenase, glutathione S transferase, microsomal monooxygenases, and NADPH oxidase.^{33,34} They also inhibit ROS generation by chelating free Fe and Cu which are involved in Haber Weiss and Fenton reactions.³⁵ There is increasing evidence that polyphenols may protect cells against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress.

Resveratrol is a nonflavonoid polyphenol present in many plant species. The most important are cacao, peanut and grape and their relative by-products (such as red wine and chocolate). It has been shown that resveratrol has anti-inflammatory and anti-oxidative effects *in vitro*.³⁶

Curcumin

Curcumin is the major component in turmeric, which may make up to 2% – 5% of the total spice in turmeric. Curcumin has been shown to have significant antioxidant activity against a variety of free radicals both *in vitro* and *in vivo*.^{37,38}

2. OXIDANTS AND ANTIOXIDANTS IN HEALTH AND DISEASE

a) Physiological roles:

Even though ROS have damaging effects under pathological conditions, they also play a significant role in the regulation and maintenance of normal processes in the organism.

1. Within the vascular structures, oxidized environment in the plasma leads to oxidation of cellular plasma membranes and cytoskeletal proteins. Oxidized proteins then stimulate mitochondrial production of ROS that will initiate signaling pathways upregulating the cellular responses which result in normal vascular diameter regulation in humans.^{39,40}
2. Oxygen sensing is another critical function of ROS in living organisms. During acute hypoxia, the mitochondrial ETC produces excess ROS which activates hypoxia inducible factor (HIF-1). HIF-1 then regulates adaptive metabolic responses that result in a normalization of oxidative stress and maintenance of redox homeostasis in humans.⁴¹ Additionally HIF-1 activity stimulates angiogenesis, which augments oxygen delivery to tissues.⁴²
3. ROS are involved in both the innate and the adaptive immune system responses. One of the first lines of defense upon exposure to invading microorganisms and other pathogens is the increased ROS production as a part of the oxidative burst in activated phagocytes.⁴³⁻⁴⁵ In addition, within the adaptive immune system excess ROS produced locally by phagocytes enhance the intracellular signal transduction pathways within the T lymphocytes and thus decrease their activation threshold.⁴⁶
4. ROS may be important in the regulation of skeletal muscle glucose uptake during contraction. However, studies on this issue are somewhat inconsistent.⁴⁷⁻⁴⁹ Muscle activity has recently been reported to affect antioxidant status.^{50,51} Exercise, whether acute or chronic, may help in maintaining redox homeostasis because it increases the antioxidant defense mechanisms, and acute heavy exercise such as cycloergometric tests, including maximal and submaximal episodes may render people more resistant

to damage caused by oxidative stress.⁵¹ On the other hand, ultra-endurance exercise was found to be related to increased ROS production and decreased antioxidant molecule levels which suggests an impaired capacity to cope with ROS.⁵² Immobilization can also cause an increase in ROS production in skeletal muscle tissue.⁴⁶

5. Cellular redox status is also important in genomic stability, regulation of transcription and signal transmission.⁵³ The deacetylase activity of sirtuins, which are members of class III histone deacetylases, depends on the cellular redox status. When oxidative stress increases, the role played by ROS in transcription regulation is of critical importance and it affects vital processes such as glucose homeostasis, cellular lifespan, inflammation, and multiple aging-related diseases.⁵⁴ Lymphocyte signal transduction cascades are affected by redox status via activity of redox-responsive transcription factors like AP-1 and NF- κ B increases. NF- κ B binds and activates the promoters of various genes. Among those genes is the gene for the protective protein thioredoxin (Trx). It is an oxidoreductase and functions together with the glutathione enzyme system to establish a reducing intracellular redox state.^{46,55}

b) Pathological actions: disease states associated with oxidative stress.

Since high levels of ROS in the body interfere with the normal physiologic processes it is not surprising that they have been implicated in a variety of disease states.⁵⁶⁻⁶² An imbalance in redox status results in damage to proteins, lipids and DNA.^{54,63} As a result of lipid peroxidation the structure of the cell membrane especially its fluidity changes. Lipid peroxidation also changes the hydrophobic properties of the cell membrane that leads to an increase in cell permeability. Protein oxidation may cause structural changes by creation of extra bonds that may result in enzyme inactivation or denaturation. Oxidative stress contributes to increased inflammation, apoptosis or necrosis of the cells as well as the disturbance of the DNA structure and stability. ROS may also cause smooth muscle hypertrophy, increased mucus secretion and fibrosis in the airways. Consequently, oxidative stress contributes to pathogenesis of many diseases including atherosclerosis, hypertension ischemia/perfusion, cancer, neurological disorders, diabetes mellitus, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, asthma and to the aging processes.⁵⁶⁻⁶²

3. EVIDENCE FOR THE PRESENCE OF OXIDATIVE STRESS IN ASTHMA

Antioxidants balance the effects of ROS and work to protect cells and tissues against the continuous production of reactive oxygen and reactive nitrogen species during normal metabolism.⁶⁴⁻⁶⁶ An imbalance of the airway reducing state is a determinant of asthma initiation and severity (Figure 2).⁶⁷⁻⁶⁹ The non-enzymatic antioxidants include mainly ascorbic acid, glutathione, albumin, alpha-tocopherol, uric acid and β -carotene.⁷⁰⁻⁷² In addition to the increased ROS/RNS (Reactive nitrogen species), asthmatic lungs have lower enzymatic antioxidants such as SODs, catalase and glutathione peroxidases as well as heme oxygenase-1, thioredoxins, peroxiredoxins and glutaredoxins.⁷³ Asthma is characterized by decreased activities of antioxidants.^{67,74,75}

Findings for transcriptional regulation of oxidative stress and signaling pathways

There is strong evidence for the presence oxidative stress in asthma.^{67,69,76} The hierarchical oxidative stress model.^{64,77} serves to unify the physiological activation of the oxidant system with the pathological actions observed at higher levels. Low level of oxidative stress leads to the activation of the transcription factor, nuclear erythroid 2 p45-related factor 2 (Nrf2), which encodes more than 200 genes. It is responsible for a wide range of anti-oxidant, anti-inflammatory and cytoprotective functions. At this stage antioxidants can restore cellular redox homeostasis. At higher levels of oxidative stimuli, however, a number of pro-inflammatory intracellular signaling cascades such as mitogen activated protein kinase (MAPK) and nuclear factor-KB (NF- KB) are activated. If the the oxidative stress continues to increase a cytotoxic response in the mitochondria ensues which finally culminates in cellular apoptosis or necrosis.⁷⁵

Transcription factors, NF-kB and activation protein-1 (AP1), are regulated and influenced by the oxidative stress and take place in the transcriptional regulation of many genes involved in oxidant stress. Reynaert et al. demonstrated that S-glutathionylation regulates activation of the NF-kB pathway^{78,79}. AP1 regulates many of the inflammatory and immune genes in oxidant-mediated diseases. Gene expression of g-GCS, the rate-limiting enzyme for the GSH synthesis, is induced by the activation of AP1.⁸⁰

Findings in the airways (Exhaled breath condensate, bronchoalveolar fluid)

Many biomarkers of respiratory inflammation and oxidative stress have been detected within **exhaled breath condensate** (EBC) samples in various inflammatory lung diseases.^{60,81}

In a systematic review for EBC studies in adult asthmatics 16 papers were evaluated by Aldakheel et al. They found that concentrations of exhaled hydrogen ions, nitric oxide, hydrogen peroxide and 8-isoprostanes were generally elevated and were related with decrease in lung function tests in asthmatic adults compared to healthy controls. The results were more often detected in patients with severe and uncontrolled asthma.⁸²

In another systematic review by Thomas et al. 46 studies were evaluated to show the relation between EBC markers and asthmatic inflammation and oxidative stress in children. The results suggested that hydrogen ions (as pH), hydrogen peroxide, and oxides of nitrogen are associated with pediatric allergic asthma and exacerbations. Additionally more complex molecules such as leukotrienes, prostaglandins, IL-4 and IL-5 are also elevated in the breath of patients with asthma.⁸³

Within the airways ROS react with lipids resulting in formation of isoprostane and ethane.^{65,84,85} 8-isoprostane, a biomarker of lipid peroxidation, was consistently found to be elevated in exhaled breath condensate of asthmatic children and adults.⁶⁵ There is also an increase in urinary levels of bromotyrosine and F2-isoprostanes in asthmatic patients.⁸⁶

Acute asthma exacerbations may lead to a decrease in glutathione levels in the exhaled breath condensate of children compared to control subjects. Furthermore, the glutathione levels in exhaled breath condensate seem to increase after oral steroid treatment compared with pretreatment levels.⁸⁷ Glutathione depletion may be related with inhibition of Th1-associated cytokine production and favors Th2 associated response.⁸⁸

In another study the level of 3-bromotyrosine was found to be 3 times higher in the bronchoalveolar lavage fluid of asthmatics compared to control subjects.⁸⁹ It is characterized as a unique product of EPO and eosinophils. Additionally, the level of 3-bromotyrosine in severe asthma patients followed in intensive care units was found to be 100 times higher than the patients hospitalized for non-asthma causes.⁹⁰ Catalase, another antioxidant enzyme, showed lower activity in bronchoalveolar lavage fluid in asthma as compared to healthy controls both in animal and human studies.⁹¹ Nitration and oxidation cause decreased catalase activity in-vivo, which identifies oxidative inactivation as the mechanism of activity loss.⁹¹

Findings in the systemic circulation and urine

Previously we evaluated the enzymatic and non-enzymatic antioxidant systems in asthmatic children.⁹² Antioxidant enzymes glutathione peroxidase and SOD, and nonenzymatic components of the antioxidant system including reduced glutathione, ascorbic acid, α -tocopherol, lycopene, and β -carotene were significantly lower in asthmatic children compared with healthy controls. Glycine and glutamine amino acids, contributing to glutathione synthesis, were significantly lower in asthmatic children. The results suggested that childhood asthma is associated with significant decreases in various components of both enzymatic and nonenzymatic antioxidant defenses.⁹²

In two studies the amount of ROS produced by neutrophils and eosinophils were correlated with the severity of hyperreactivity in the airways.^{68,93} Furthermore, Jatakanon et al showed the relation of myeloperoxidase mediated oxidation with asthma severity.⁹⁴

Wu et al. applied a localized allergen challenge by fiberoptic bronchoscopy to asthmatics and control subjects. Two to three fold elevations in chlorotyrosine have been detected from allergen challenged subsegments in asthmatic patients.⁸⁹ Moreover, children with asthma had increased levels of malondialdehyde, a marker of lipid peroxidation, and lower than normal levels of glutathione. Additionally, as the malondialdehyde levels got higher the probability of severe asthma increased.⁶¹

Experimental Findings

The redox thiol/dithiol imbalance causes a change in intracellular copper-zinc SOD (CuZnSOD) in asthma and CuZnSOD becomes susceptible to auto-inactivation by hydrogen peroxide.^{69,70,74,95} Nitration of tyrosyl residues in the protein leads to the decrease in mitochondrial manganese SOD (MnSOD) activity.^{69,96} In a mouse model of asthma it was shown that the CuZnSOD transgenic mice have less airway inflammation and hyperreactivity in comparison to wild-type mice.⁹⁷ In contrast to the classical knowledge that increased ROS in asthma is usually attributed to leukocyte activation, an asthma murine model showed that there is more ROS activity in the mitochondria of airway smooth muscle.⁹⁸ In support of this, asthmatic patients given coenzyme Q, a component of mitochondrial electron transport chain and a mitochondrial antioxidant, recovered SOD activity to healthy, nonasthmatic levels.⁹⁹

Catalase activity in bronchoalveolar lavage fluid is lower in asthma as compared to healthy controls both in animal and human studies.⁹¹ Nitration and oxidation cause a decreased catalase activity in-vivo, which identifies oxidative inactivation as the mechanism of activity loss.⁹¹

4. BIOMARKERS FOR OXIDATIVE STRESS IN ASTHMA

The labile nature of the molecules of oxidative stress makes them difficult to measure. However stable end-products of oxidative pathways may be used to determine the level of oxidative stress. Many direct or indirect markers of oxidative stress such as bromotyrosine, malondialdehyde, isoprostane, thiobarbituric acid, glutathione disulfide have been detected in urine, plasma, sputum and BAL fluid of patients with asthma and the level of the markers correlated with the clinical output and severity of the disease⁶⁸⁻⁷⁰. A non-invasive way of analysis, exhaled breath condensate collection, has allowed direct measurements of pH changes, H₂O₂ and the measurement of several indirect byproducts of oxidation like 8-isoprostane and ethane.^{82,83} Currently, the presence of high levels of urine bromotyrosine is a promising noninvasive biomarker of oxidative stress for clinical use in asthma patients.

Bromotyrosine

Eosinophils are important cellular components of atopic and Th2 driven asthma. One of the enzymes released by activated eosinophils is the eosinophil peroxidase (EPO).¹⁰⁰ Myeloperoxidase, a member of mammalian peroxidase family like EPO, is present in neutrophils and monocytes, and released when the cells are activated.⁵⁹ While MPO uses mainly chloride; EPO uses bromide, but cannot use chloride as a substrate. In other words, chlorotyrosine is specific for MPO, and bromotyrosine is selective for EPO in vivo.^{89,90} Bromotyrosine level is higher in asthmatic airways and it increases with asthma exacerbations.^{25,89} Bromotyrosine is a stable end product and can be quantified in urine where it is excreted, and may be used as a biomarker for the activated eosinophils.¹⁰¹ Urine bromotyrosine levels increase during asthma exacerbation,¹⁰¹ and this increase is related with a poor asthma control, or at risk of an asthmatic exacerbation.¹⁰²

5. IS THERE A ROLE FOR ANTI-OXIDANTS IN THE TREATMENT OF ASTHMA

Even though it is apparent that treatment modalities targeting the oxidant stress has a great potential for the treatment of many diseases including asthma, so far only few of these compounds have been studied in large clinical trials and generally human studies have been disappointing and have failed to reveal a clear benefit.

a) Observational Studies

In 2016 Garcia-Larsen provided a summary of systematic reviews on the relation between asthma and dietary intake. A total of 7 high quality systematic reviews were evaluated.¹⁰³ One systematic review analysed the evidence from eight observational studies on various measures of Mediterranean diet adherence in and various outcomes of asthma (wheeze) and severity in children.¹⁰⁴ In this review, it was found that Mediterranean diet is associated with lower occurrence of the current wheeze, current severe wheeze and asthma ever. With regard to antioxidant vitamins, Allen et al in 2009 reviewed 40 studies on antioxidants and asthma outcome measures in a meta-analysis. Studies were mostly cross sectional and case control and there were no clinical trials. Dietary vitamin A intake was significantly lower in people with asthma than in those without asthma and in people with severe asthma than in those with mild asthma. In the lowest quartile of the dietary vitamin C intake there was a 12% increase in asthma risk. Vitamin E intake was unrelated to asthma but was significantly lower in severe asthma than in mild asthma.¹⁰⁵

Nurmatov et al. published a systematic review to document the effect of diet in preventing asthma in children. A total of 62 studies which comprised of 21 cohorts, 15 case control and 26 cross sectional studies were evaluated. Serum vitamin A was found to be lower in children with asthma compared with controls ([OR], 0.25; 95% CI, 0.10-0.40). Additionally high maternal dietary vitamin D and E intakes during pregnancy were found to be related with the lower frequency of wheezing. Mediterranean diet could be of importance for persistent wheeze and atopy. Seventeen of 22 fruit and vegetable studies reported beneficial associations with asthma and allergy. Results were not supportive for any outcomes in relation to vitamin C and selenium.¹⁰⁶

In another systematic review conducted by Patelarau et al, 18 studies were evaluated to evaluate the relation between antioxidant levels in pregnant woman and allergic diseases in their infants. The review included 5 prospective cohort studies, 4 cross-sectional studies, and 9 case-control studies. Eight studies reported an important association between antioxidant status and asthma onset during childhood.¹⁰⁷

b) Interventional Studies

Despite the suggestion by epidemiological studies that patients with low dietary intake of vitamin C, E and other antioxidants have usually more symptoms, the results of the clinical studies have been largely discouraging.^{105,108} Interestingly, a recent systematic review for the prevention of cancer and cardiovascular diseases by the supplementation of dietary antioxidants revealed an increase in the cancer incidence and mortality which suggested that vitamin supplements may increase the oxidant stress and may be harmful.¹⁰⁹

In a randomized double blind placebo controlled trial by Morrison et al 11 healthy young men were given either vitaminC (2x500mg/day) and vit E(400IU/day)) or placebo. Following acute exercise, vitamin C and E supplementation did not attenuate skeletal muscle oxidative stress or the increase in gene expression of mitochondrial biogenesis markers. In contrast, however, vitamin C and E supplementation did attenuate some of the cellular adaptations in skeletal muscle (mitochondrial transcription factor A and SOD) following 4 weeks of endurance training.¹¹⁰

In a recent randomised placebo controlled trial, Suduni et al investigated the effect of broccoli sprout rich in sulforaphane, an effective enzyme which is a potent inducer of phase II enzymes, which play a critical role in preventing oxidative stress. 40 asthmatic adults were randomised to get either 100 gram per day broccoli sprout or placebo for 3 days. Exhaled Nitric Oxide (FeNO), FEV1%, nasal epithelial and peripheral blood mononuclear cell gene expressions, inflammatory and oxidative stress biomarkers, and symptoms were assessed before and after ingestion of the sprouts. Despite an increase in the serum levels of sulforaphane, broccoli sprouts did not affect eosinophilic pulmonary inflammation, inflammatory and oxidative stress biomarkers, clinical features of asthma or induction of antioxidant genes among atopic asthmatics.¹¹¹

In another study on diabetic patients, the investigators found that daily supplementation with vitamin C and vitamin A for four weeks not only failed to improve the insulin responsiveness but also decreased the expression of ROS sensitive transcriptional regulators and molecular mediators of ROS defense (SOD 1 and 2; glutathione peroxidase).¹¹² In this study, antioxidant supplement seems to have blocked the endogenous defenses rather than counteract the oxidant stress. This emphasizes that exogenous antioxidants need to block the oxidant pathways without suppressing the endogenous antioxidant mechanisms.

Romieu et al. performed a double blind placebo controlled trial to see the effect of antioxidants in children exposed to high levels of air pollution. A total of 158 asthmatic children were randomised and were given either a daily supplement of vitamins (50 mg/day of vitamin E and 250 mg/day of vitamin C) or a placebo for 12 weeks. Spirometry tests results were used as an outcome measure. Environmental ozone levels a day before testing were inversely associated with lung function in children with moderate/severe asthma. For each increase of 10 ppb of ozone, forced expiratory flow from 25% to 75% of vital capacity decreased by 13.3 mL/s, FEV1 decreased by 4.59 mL/s, and peak expiratory flow decreased by 15.0 mL/s in the placebo group. However there was no association in the supplement group.¹¹³ These findings support the idea that antioxidant supplementation may change the effect of ozone exposure on the respiratory tract of children with asthma.

Wood et al. randomised adult asthmatics either to a low or high antioxidant diet for 14 days and then gave 45 mg lycopene (tomato extract) to half of the low antioxidant diet group for 14 weeks or until an exacerbation occurred. Compared to the high antioxidant intake group the low antioxidant intake group showed a 2.26 fold increase in asthma exacerbation risk. Within the low antioxidant intake group there was no difference in systematic or airway inflammation in relation to the lycopene intake.¹¹⁴

In another randomised controlled trial, 7 to 10 year old children were given 200 mg/day vitamin C for 6 weeks. Childhood asthma control tests (C-ACT) and FEV1 values were used as the outcome measures. The effect of Vitamin C on asthmatic children was heterogeneous. The effect of Vitamin C on C-ACT scores varied with age and baseline C-ACT scores and effects on FEV1 levels varied with age and exposure to the dampness.¹¹⁵

Thiol antioxidants, precursors of glutathione, are the most commonly used antioxidant therapeutics. N-acetyl cysteine (NAC) is the most frequently studied thiol precursor. NAC was found to suppress airway inflammation and hyperreactivity in animals¹¹⁶ and inhibit ROS

production in human peripheral blood eosinophils¹¹⁷. In animal models oral NAC exerted an antioxidant protective effect and attenuated pulmonary inflammation.^{116,118} According to a meta analyses, NAC was able to decrease the exacerbation rate in chronic obstructive pulmonary disease (COPD) patients when used as an add on therapy at 1200 mg daily.¹¹⁹ In contrast, in a randomized controlled study, NAC was found to be ineffective to prevent the decrease in lung function and exacerbations in COPD patients.¹²⁰ Another study showed that NAC produced no significant benefit when added to conventional treatment during an asthma attack.¹²¹ The lack of clear benefit in human studies may be related to the relative instability of NAC in-vivo.¹²²

Resveratrol, a phytoalexin found in seeds of grapes has both antioxidant and anti-inflammatory capacity. It affects lung epithelial cells and induces GSH synthesis and attenuates GSH depletion in the presence of oxidative stress. Resveratrol decreases IL-1 and TNF-alpha expression¹²³ and reduces inducible nitric oxide synthase (iNOS) expression and COX-2 mRNA in human lung epithelial cells.¹²⁴ In a previous study, resveratrol blocked the release of both IL-8 and granulocyte macrophage colony stimulating factor (GM-CSF) from alveolar macrophages of COPD patients both at basal state and after stimulation with IL-1 β and cigarette smoke.¹²⁵

A limited number of clinical trials of flavonoids for asthma have been performed. In a randomized, double-blind, placebo-controlled, crossover design study of 26 patients with asthma were given 1 mg/lb/day (maximum 200 mg/day) pycnogenol, a mixture of water-soluble bioflavonoids which is extracted from French maritime pine, for four weeks. The patients treated with pycnogenol had a significant reduction in serum leukotriene levels compared to the placebo group.¹²⁶ Lau et al performed a randomized, placebo-controlled, double-blind study involving 60 asthmatic children aged 6–18 years and gave pycnogenol for 3 months. The pycnogenol group showed significantly greater improvement in peak expiratory flows expressed as percent of predicted values and asthma symptom scores starting with the first month of treatment. There was also a significant reduction in urinary leukotrienes and a reduction in or discontinuation of the use of rescue inhalers for the pycnogenol group.¹²⁷

6. CONCLUSION

It is clear that asthma is associated with oxidant stress that is closely related to disease airflow obstruction, airway hyperreactivity and remodeling. Even though modulation of oxidative stress with dietary supplements offers great promise in the treatment of asthma, so far no compound has proven to be clinically useful in the modulation of the disease. The ideal compound should block the formation and/or effects of the oxidant without compromising the physiologic functions as well as without blocking the endogenous antioxidants. Application of an individualized precision medicine approach may uncover the phenotype of patients that benefit most from an antioxidant intervention.

TABLES

Table 1. Major sources of oxidants

ENDOGENOUS SOURCES OF OXIDANTS	EXOGENOUS SOURCES OF OXIDANTS
Mitochondria	Cigarette smoke
Cytochrom p450	Ozone
Peroxisomes	Hyperoxia
NAD(P)H oxidase	Heavy metals
Xanthine oxidase	Ionizing radiation
Haber Weiss and Fenton reactions (Reaction of metal ions)	Ultraviolet light
Myeloperoxidase (MPO)	

Table 2. Enzymatic and non-enzymatic Antioxidants

NAME	ACRONYM	FUNCTION
Enzymatic Antioxidants		
Superoxide dismutase	SOD	Neutralizes superoxide anion. Prevents lipid peroxidation
Catalase	CAT	Conversion of H ₂ O ₂ to water and molecular oxygen
Glutathione peroxidase	GSH-Px	Catalyzes the conversion of H ₂ O ₂ or organic peroxide to water or alcohol
Heme oxygenase	HMOX	Degrades heme to biliverdin and subsequently biliverdin, which are exert antioxidant properties. Decreases superoxide and ROS formation
Thioredoxin	TRX	The Trx system provides the electrons to thiol-dependent peroxidases (peroxiredoxins) to remove reactive oxygen and nitrogen species
Peroxiredoxin	PRX	Scavenges H ₂ O ₂ , ROOH, and ONOO ⁻
Glutathione transferase	GST	Catalyzes the conjugation of electrophilic substrates to glutathione (GSH) and reduction of hydroperoxides
Glutathione reductase		Catalyzes NADH-dependent reduction of oxidized glutathione (GSSG) to GSH
Non Enzymatic Antioxidants		
All trans retinol	Vitamin A	Inhibits lipid peroxidation
Ascorbic acid	Vitamin C	Neutralizes hydrogen peroxide, superoxide and hydroxyl radicals
Alpha tocopherol	Vitamin E	Scavenges lipid peroxyl radicals via hydrogen atom transferring
Beta carotene		Quenches singlet molecular oxygen
Glutathione		Co-factor for several detoxifying enzymes. Scavenges hydroxyl radical and singlet oxygen directly, regenerate Vitamins C and E
Melatonin		Neutralizing HO [•] and peroxyl radicals, CO ₃ ^{•-} , NO ₂ [•] , O ₂ ^{•-} , and HOCl.
Polyphenols		Inhibits Haber Weiss/Fenton reactions by chelating free Fe and Cu. Inhibits oxidation of lipids and proteins by accepting an electron to form relatively stable phenoxyl radicals.
Curcumin		Free radical scavenging
Cu, Mn, Se, Zn		Co factors of antioxidant enzymes such as SODs and GTPx

FIGURE LEGENDS

Figure 1. The major enzymatic antioxidants of the lungs.

Figure 2. An imbalance of the airway reducing state is a determinant of asthma initiation, airway hyperreactivity and remodeling.

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