

The Role of Oxidative Stress and Lipid Peroxidation in Allergic Asthma

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Published Date: January 03, 2017

ABSTRACT

Asthma is a heterogeneous disease with genetic and environmental components, described as chronic inflammation and long term irreversible remodeling of the airways associated to an inappropriate inflammatory response. Asthma is also classified in two main phenotypes: atopic and non-atopic asthma depending if it is related to allergies or not.

Oxidative stress is related to airway inflammation and its endogenous and exogenous reactive oxygen and nitrogen species are associated to airway inflammation and consequently to asthma and its severity. Lipid peroxidation is a form of oxidative stress that involves all oxidation of fatty acids by enzymatic and non-enzymatic reactions. Non-enzymatic reactions occur between a reactive species and a fatty acid which culminates into stable bioactive products through a chain of reactions. Oxidation of arachidonic acid is a fundamental enzymatic reaction that leads to the formation of bioactive metabolites such as prostaglandins and leukotrienes with an important role on asthma.

With this review, it is intended to summarize the mechanisms of oxidative stress, lipid peroxidation and the roles of each generated metabolite or group of metabolites on the inflammatory response and asthma.

Keywords: Asthma; Oxidative stress; Lipid peroxidation; Arachidonic acid; Metabolomic

INTRODUCTION

Asthma is a syndrome [1] or heterogeneous disease with different phenotypes essentially defined by age and triggers [2,3]. This disease affects more than 334 million people of all ages and ethnicities all over the world affecting also their families, health systems and government [4,5].

It is described as a chronic inflammation and long-term irreversible remodeling of the airways [1,6]. There is evidence that asthma is somehow related to the generation of reactive species [7-10]. The inflammatory cells, the airway tissue cells and the involved mediators are the main sources of oxidative stress in asthma producing high levels of reactive oxygen (**ROS**) and nitrogen species (**RNS**) that play an important role in this process [11]. Asthma is also connected to lipid peroxidation that involves oxidation of fatty acids, namely the arachidonic acid, occurring in vivo either by enzymatic or non-enzymatic reactions [12].

This work provides an overview about the role of the oxidative stress in asthma and the biological mechanisms underlying this process.

BRIEF DEFINITION AND DIVERSITY OF ASTHMA

Asthma is a syndrome [1] or heterogeneous disease with both genetic and environmental components. Genetic components are defined by genes associated to asthma development that were previously identified [13,14], atopy by itself is a genetic predisposition to an IgE mediated response development – mediated to the common aeroallergens [15], in addition, asthma is not equally distributed between the sexes being probably due to genetic factors [16]. Environmental components are related to the exposure of the individual to aeroallergen [17], viral or bacterial infections [18], active or passive smoking which leads to the development or complication of asthma [19], nutrition as a way to treat or prevent asthma [20], exercise that leads to the manifestation of symptoms in most patients [21], climatic conditions, like extreme temperatures and high humidity are associated to asthma activity and development of asthma exacerbations [22].

Described as chronic inflammation and long-term irreversible remodeling of the airways, asthma is also defined by the history of respiratory symptoms such as: dyspnea, wheezing, breathless, chest tightness and cough that vary over time and in intensity [1,3,6,23] - associated to one or more triggers with an additive or synergetic effect on the development of mild symptoms or exacerbations [23], culminating in an inappropriate inflammatory response: contraction of the smooth muscle that constricts the airways and consequent narrowing and obstruction of the lumen due to airway wall edema and the excess of mucus in the airways, associated to an increase of the mucin, a glycoprotein that is the main constituent of mucus [24].

In 1947, Francis Rackman classified asthma in two clinically distinct phenotypes: atopic or extrinsic asthma and non-atopic or intrinsic asthma [2]. The atopic phenotype is highly related to allergies, characterized by airway hyperresponsiveness, infiltration of the mucosa with

eosinophils and T lymphocytes type 2 (**Th2**), circulating specific immunoglobulin E (**IgE**) and positive skin prick tests to common allergens (e.g. pollens, mold, mites and animal epithelia). It is more common, contributing with about 73% of the asthmatic cases and arises earlier, before 30 years old. Its diagnosis is relatively simple and generally based on clinical and familiar history of the patient. It may complicate due to infections and is also associated to asthmatic bronchitis, cough and rhinitis that can lead to a severe form of asthma. Non-atopic asthma is hard to diagnose once its patients don't have a clinical familiar history of allergies, having its origin in factors that are not related to allergies such as bacterial origin. 'Polypoid sinusitis', dyspnea and negative results on skin prick tests are common in these individuals. The total IgE concentration range in their serum is normal without any evidence of specific IgE against common aeroallergens. They show sensitization to aspirin more frequently, and psychosomatic exhaustion factors should be considered [2,17,25,26]. It affects about one fourth (i.e. 27%) of the asthmatic population. These individuals tend to be female and older than the atopic asthmatics (> 30-40 years old).

According to Bacharier et al. [3], the different phenotypes of asthma are defined by age and triggers. Symptoms persistence (i.e. wheezing) in infant children (0-2 years old) is an important indicator of severity. In preschool children (3-5 years old) symptoms persistence during the last 12 months is a differentiator indicator of asthma phenotype. Depending on the symptoms, asthma can be classified as virus- (more common at this age), exercise- or allergen-induced. In school-age children (6-12 years old), although the symptoms are comparable to the symptoms of the preschool children, in addition to virus-induced, the allergen-induced asthma is more common and visible. Seasonality may become evident and severity can become an issue because the lack of response to therapy and lung function. In adolescence (13-16 years old), atopic asthma has more new cases than remissions and non-atopic atopic can have its beginning at this age. Some issues like smoking, the follow-up of the therapy, and the change of the pediatric to another physician have its beginning at this age. In 2004, de Blic et al. [27] have suggested that severe asthma can be considered as a unique phenotype, that it is age-dependent, related to persistence and to lack of response to the therapy and that it can be measured by the frequency of symptoms and lung function.

OXIDATIVE STRESS

Oxidative stress is defined by the damage that occurs in the body when reactive species outgrow the antioxidant defenses of the body [28].

The reactive oxygen species (**ROS**) and the reactive nitrogen species (**RNS**), either endogenous or exogenous, play an important role in airway inflammation and are determinant compounds in the severity of asthma. An excess of this reactive species in the organism lead to deep structural cellular damage through the oxidation of the main biomolecules: lipids, proteins and DNA [29]. According to the hierarchical model of oxidative stress, when the organism is exposed to low levels of environmental oxidative stress, it is able to recover cellular oxidation-reduction (**redox**)

homeostasis through the production of different enzymatic antioxidants such as catalase (**CAT**), superoxide dismutase (**SOD**)-3, heme oxygenase-1 (**HO-1**), glutathione-S-transferases (**GST**), NAD(P)H-quinone oxidoreductase, glutathione peroxidase (**GPx**) and glucuronosyltransferase-1a6 (**UGT-1a6**) induced through nuclear erythroid 2 p45-related factor 2 (**Nrf-2**) responsible for activating the transcription of more than 200 genes. Because of its ability to respond to low levels of oxidative stress, Nrf-2 may be a determinant factor of the susceptibility of the individuals to develop asthma as a response to environmental stimuli [30]. When exposed to intermediate levels, which overwhelmed the antioxidant capacity of the organism, other potentially pro-inflammatory pathways are developed - e.g. mitogen-activated protein kinase (**MAPK**) and nuclear factor- κ B (**NF- κ B**) - leading to the expression of cytokines, chemokines, and adhesion molecules that perform an important role in airway inflammation and consequently in asthma per se. An increase to high levels of oxidative stress can lead to a mitochondrial cytotoxic response resulting in cellular apoptosis or necrosis [11,31].

Endogenous and Exogenous Reactive Species and Their Biological Effects on Asthma

The inflammatory cells (mast cells, eosinophils, macrophages and neutrophils) and the airway tissue cells (epithelium and smooth muscle) produce ROS after their activation by a variety of stimuli - endogenous ROS (Figure 1) [11,32,33]. For example, the superoxide anion ($O_2^{\bullet -}$) is produced by the nicotinamide adenine dinucleotide phosphate (**NADP**) oxidase-dependent complex, by the cytosolic xanthine oxidase system and by the electron transport in mitochondria. This species is then converted to hydrogen peroxide (H_2O_2) enzymatically or reacting with itself (spontaneously). Both $O_2^{\bullet -}$ and H_2O_2 species can be catalyzed by traces of transition ions, such as iron present in the body, into hydroxyl radical ($OH^{\bullet}$), a much more reactive species than $O_2^{\bullet -}$ and H_2O_2 . In fact, much of the damage caused by $O_2^{\bullet -}$ and H_2O_2 is due to the formation of $OH^{\bullet}$. For example, Fe^{3+} is reduced to Fe^{2+} (Haber-Weiss reaction) and Fe^{2+} is then oxidized into Fe^{3+} leading to the formation of $OH^{\bullet}$ and OH^- from H_2O_2 (Fenton reaction) [34]. Another way to the formation of $OH^{\bullet}$ is through the formation of hypohalides (e.g. hypochlorous acid - HOCl and hypobromous acid - HOBr) that can occur due to the reaction between H_2O_2 and halides (compounds that result from the reaction between a halogen and another species) catalyzed by peroxidases present in inflammatory cells such as eosinophils, neutrophils and monocytes. Subsequently, the hypohalides react with $O_2^{\bullet -}$ forming $OH^{\bullet}$ [35-37].

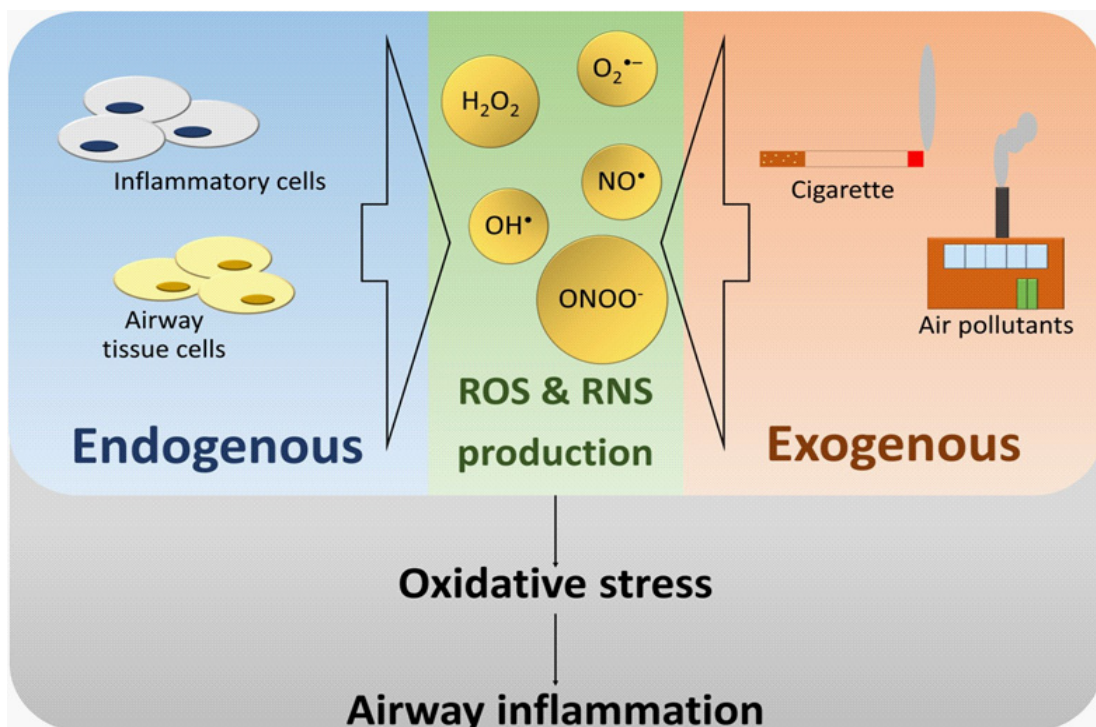


Figure 1: Endogenous and exogenous reactive species role in airway inflammation. Oxidative stress is caused by the excess of both endogenous (produced by inflammatory cells and airway tissue cells) and exogenous (produced by air pollution, cigarette, etc.) reactive species that lead to airway inflammation [10,11,32,33,37,45,52,53]. Abbreviations: RNS, reactive nitrogen species; ROS, reactive oxygen species.

The main RNS produced in the organism is $NO^{\bullet}$ produced by nitric oxide synthases (**NOS**). These enzymes catalyze the conversion of L-arginine into L-citrulline with the generation of $NO^{\bullet}$ [38]. In a normal situation, NO is constitutively produced in small amounts by isoenzymes NOS1 (neuronal) and NOS3 (endothelial), which are assumed to play a regulatory role in neurotransmission and blood flow respectively, or in large amounts when induced by cytokines and bacterial products on inflammation or infection (**NOS2**) [10,39]. There is evidence that asthmatic patients show increased levels of NO in the exhaled breath essentially due to an NOS2 overexpression in the endothelial cells of the lungs and respiratory tract [40] although NOS1 and NOS3 appear to be involved [41, 42]. In this regard, measurement of $NO^{\bullet}$ levels in the exhaled air (**FENO**) might be an important biomarker once most patients with the mild form of the disease do not show spirometric deviations [43]. This increased expression of NOS2 can be reduced by corticosteroids in asthmatic patients [44] but no $NO^{\bullet}$ removal system is known to best of our knowledge [9].

The $O_2^{\cdot-}$ can also react with $NO^{\cdot}$ forming peroxynitrite (**ONOO $\cdot$**) which has the power to nitrate proteins, more precisely tyrosine [10,45]. In atopic asthmatics, high levels of nitrotyrosine, a product of nitration on tyrosine, were detected [46]. In COPD patients, the consequent formation of peroxynitrite decrease the concentration levels of NO in the airways [39].

The reaction between radical species and lipids can culminate in the formation of isoprostanes and ethane [29]. Higher values of isoprostanes (i.e. 8-isoprostane) were detected in either the exhaled breath [47,48], urine [49] or plasma [28] of asthmatic patients being an *in vivo* way of accessing diseases related to oxidative stress such as asthma, diabetes and cardiovascular diseases [12]. Other products of radical action are also indicators of the severity of asthma, including 3-bromotyrosine, 3-chlorotyrosine and malondialdehyde, a marker of lipid peroxidation. Ercan et al. (2006) obtained increased levels of melondialdehyde and lower levels of glutathione in a study performed with asthmatic patients [50]. In the same way, bronchoalveolar lavage (**BAL**) of asthmatic patients presented more than a 10-fold increase in 3-bromotyrosine levels and a 2 to 3-fold increase in 3-chlorotyrosine when compared to healthy subjects after allergen challenge [51].

The exposure to ultrafine particles (smaller than 100 nanometers) present in the air, can directly reach the mitochondria, damaging them and making it difficult for the lungs to deal with the oxidative stress [52]. In addition to air pollutants, cigarette smoke and its more than 4000 compounds distributed throughout the gaseous phase, water and tar phase contribute largely to the oxidative stress. High concentrations of $O_2^{\cdot-}$ and $NO^{\cdot}$ are present in the gaseous phase of the smoke. The tar phase has organic radical species that react with the molecular oxygen (**O $_2$**) present in the air that we breathe and forms $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$, besides being a powerful chelating agent of iron and generating H_2O_2 continuously. The aqueous phase of cigarette smoke also contributes since its compounds may undergo redox reactions in the lung epithelium (Figure 1) [11,37,53].

Lipid Peroxidation

One of the consequences of the oxidative stress is lipid peroxidation and it involves all oxidation of fatty acids occurring *in vivo* either by enzymatic reactions or by non-enzymatic reactions (Figure 2) [12].

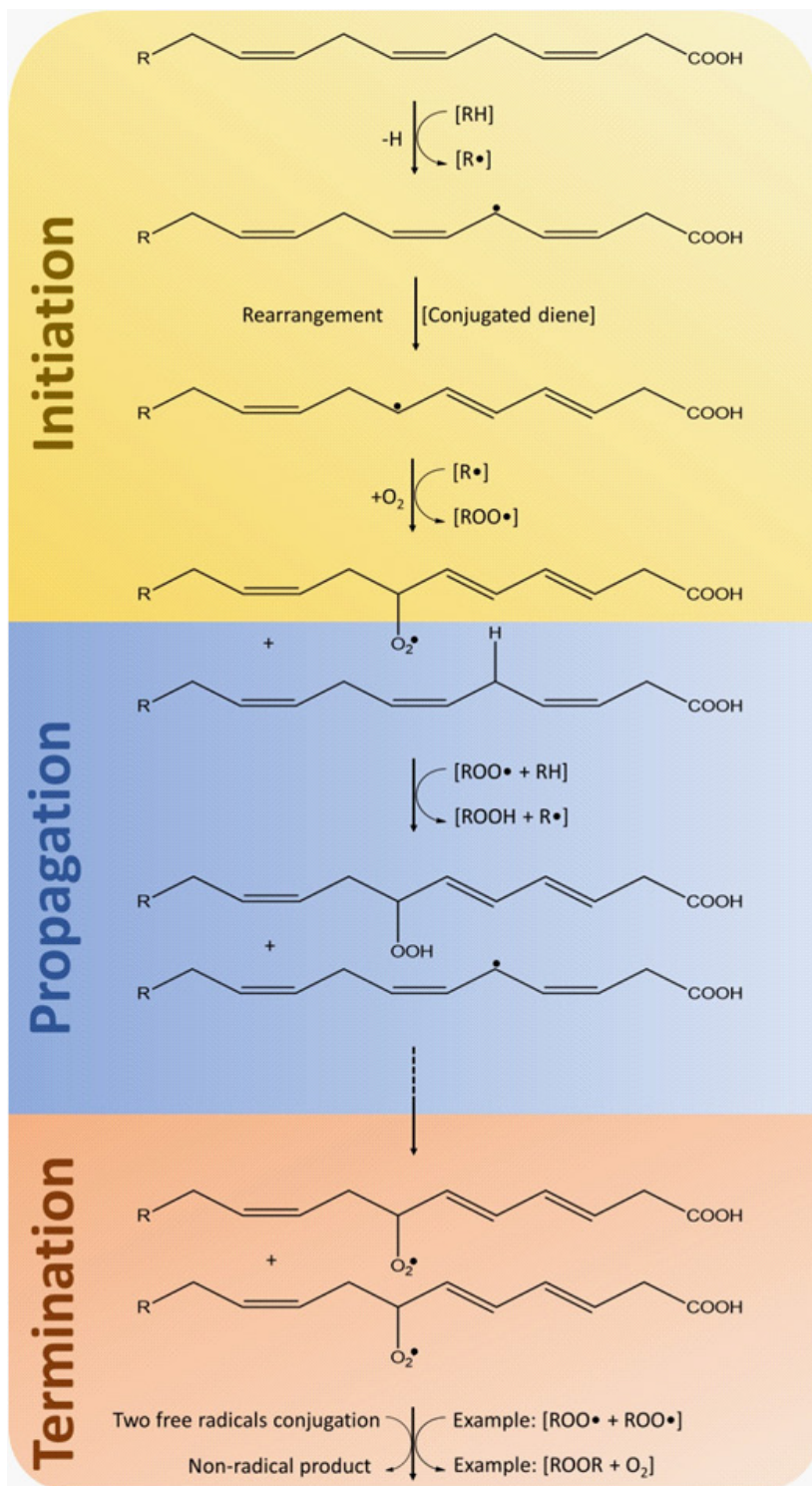


Figure 2: Basic scheme of non-enzymatic lipid peroxidation of a fatty acid [12,54].

Lipid peroxidation, which occurs through non-enzymatic reactions, is a complex process essentially divided into three phases: initiation, propagation and termination [54].

The initiation of the process occurs when a hydrogen is abstracted or a radical is added to a polyunsaturated fatty acid (**PUFA**) resulting in oxidative damage. PUFAs are more fragile than saturated fatty acids since they have double bonds adjacent to a methylene group. The C-H bond of this methylene group is weak and the hydrogen is then susceptible of being abstracted. The resulting molecule undergoes rearrangement forming a conjugated diene which combines with oxygen forming a peroxy radical. This species also has the ability to abstract one hydrogen from another PUFA. The radical centers formed have great affinity for O₂ forming different peroxy radicals. This reaction can be catalyzed by metal complexes forming alkoxy radicals (**RO•**) and OH• which also participate in the lipid peroxidation chain reaction by abstracting a hydrogen from the PUFAs. In this way, it is only necessary a starting point for many PUFAs being converted to peroxy radicals (**ROO•**) initiating, thus, the propagation of this chain reaction. The resulting radical fatty acids are stabilized by rearrangement into conjugated dienes which preserve the most stable products such as hydroperoxides (**ROOH**), alcohols, aldehydes and alkanes. The free radical chain propagates until two free radicals conjugate to terminate the chain or until they find an endogenous or exogenous antioxidant that breaks the propagation [12,54].

The resulting oxidized lipids have bioactivity as vasoconstrictors, vasodilators, bronchoconstrictors and may induce the characteristic inflammation of asthma. In addition, they have the ability to change the membrane fluidity and consequently its associated enzymes and receptors [12].

The enzymatic reactions that lead to the oxidation of arachidonic acid are mediated by cyclooxygenases and lipoxygenases and lead to the formation of bioactive metabolites such as prostaglandins (**PGs**) - eicosanoids containing a 5 carbon ring PGs - and leukotrienes (**LTs**) - eicosanoids containing 3 conjugated double bonds (trienes) [12,55-57]. These metabolites are powerful mediators of inflammation and are directly related to atrophy and weakening of airway muscles in chronic respiratory diseases [56].

The cascade of arachidonic acid (Figure 3) begins with its hydrolysis under the action of phospholipase A₂. The arachidonic acid is then converted by the enzyme 5-lipoxygenase (**LO**) and 5-LO activating protein (**FLAP**) into 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and then to LTA₄. This LT is then metabolized into lipoxins (**LXs**), LTB₄ or LTC₄, a cysteinyl-LT (**cys-LTs**) from which other 2 cys-LTs originate in the cells where it is formed - LTD₄ and LTE₄ [55,56].

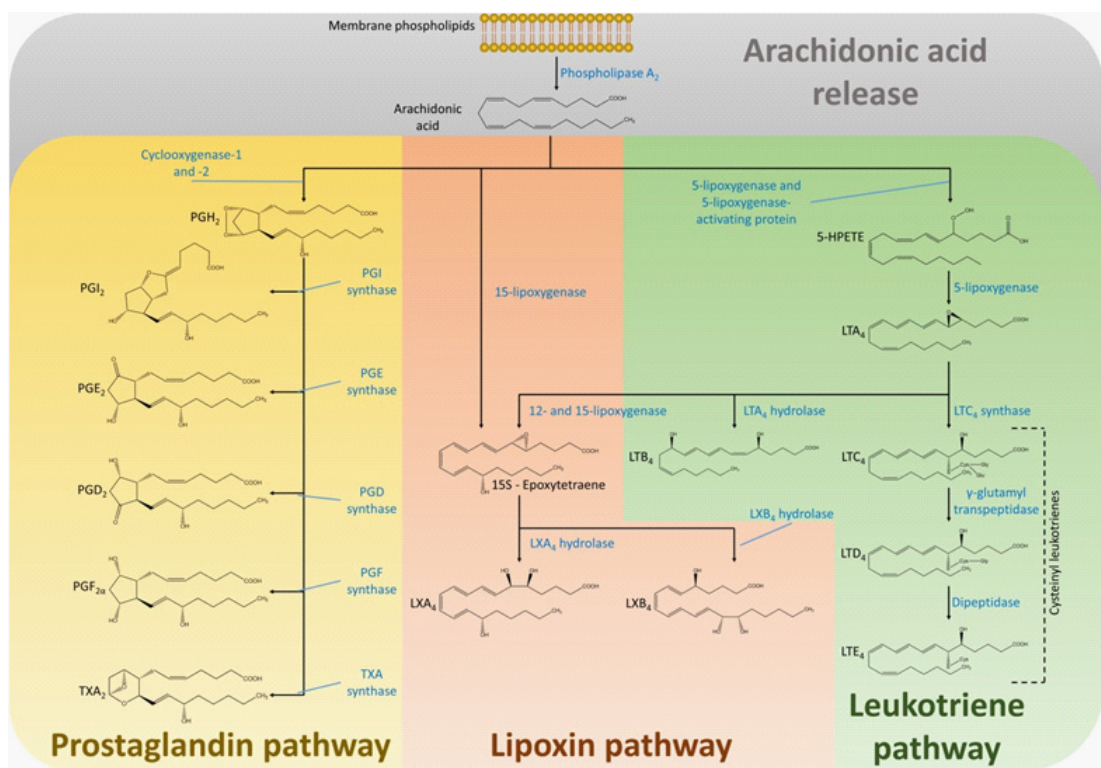


Figure 3: Arachidonic acid cascade that lead to the formation of leukotrienes, lipoxins and prostaglandins. An enzymatic lipid peroxidation [55-57, 63]. Abbreviations: 5-HPETE, arachidonic acid 5-hydroperoxide; LX_X, lipoxin X, LTX_X, leukotriene X, PGX_X, prostaglandin X, TX_X, tromboxane X.

The LTB₄ is a powerful pro-inflammatory chemoattractant which main target are the leukocytes. It activates the degranulation and release of mediators, enzymes and superoxides by the neutrophils. It increases their adhesion to the endothelium and chemotaxis, the selective recruitment into the lungs, and the activation of these leukocytes. It is involved in inflammatory pain by neutrophil dependent processes. It also acts as a ligand for the peroxisome proliferator-activated receptor-α, a transcription factor that plays a key role in the peroxidation of fatty acids [58]. This eicosanoid can be detected in an increased concentration in biological fluids such as urine of asthmatic patients [59].

Cys-LTs, are also powerful pro-inflammatory mediators, about 100-1000 times more powerful than histamine. They are chemoattractants of eosinophils, vasoconstrictors and increase the vascular permeability allowing the migration of macromolecules from the plasma to airway edema, characteristic of asthma. Furthermore, they stimulate mucus secretion and inhibit mucociliary clearance [58]. The LTE₄ may be a biomarker of asthma severity [48] once it is a biomarker of the total cys-LTs in urine [60-62].

The lipoxins such as LXA₄ and LXB₄ are distinct eicosanoids because they have a 4-bonded conjugated system (tetraene) and are the product of the action of 15-LO and/or 12-LO. Its main functions are cell regulation, inflammatory inhibition in the lungs, increased phagocytosis of macrophages, resolution of pulmonary edema, inhibition of cell proliferation, cyclooxygenase (COX)-2 activity and angiogenesis [55, 56, 63].

PGs are formed by the metabolization of arachidonic acid catalyzed by COX-1 and -2 in PGH₂, an unstable PG, rapidly converted by terminal synthases into bioactive PGs - PGE₂, PGI₂, PGF_{2α}, PGD₂ and thromboxane A₂ (TxA₂). The PGE₂ and PGD₂ can be recruited by inflammatory cells (e.g. neutrophils, macrophages and mast cells). During an inflammatory response, the level and profile of prostaglandin production changes from very low values in non-inflamed tissues to very high values due to the differential expression of the enzymes in inflamed tissues [56, 57, 64].

The PGD₂ is the most abundantly produced eicosanoid and plays an important role either in inflammation or in the homeostasis of the organism. For example, in the brain, it is involved in the regulation of sleep and other activities of the central nervous system like the perception of pain. It has long been associated with inflammation and atopy despite, in some cases, perform anti-inflammatory functions. In the inflammatory response, it is essentially produced by mast cells and leukocytes such as DCs and Th2 suggesting their role in the development of the antigen-specific response of the immune system. The PGD₂ can subsequently be metabolized to PGF_{2α}, 11β-PGF_{2α} and PGs of the J series (e.g. PGJ₂). About 150 times higher values were detected after an allergic reaction in the BAL fluid of asthmatics. It was observed bronchoconstriction and eosinophilic infiltration in tests with this PG. The pro-inflammatory effects of this PG are mediated by the DP1 and DP2/CRTH2 receptors. The DP1 receptors are expressed in the bronchial epithelium and are mediators of the production of chemokines and cytokines that recruit lymphocytes and eosinophils which in turn lead to inflammation and hyperreactivity of the airways, characteristic of asthma. Moreover, the activation of DP1 receptors also leads to the reduction of eosinophils in allergic inflammation. The DP2/CRTH2 receptors contribute to the pathogenic response by controlling cell movements. The PGD₂ recruits the Th2 and eosinophils directly through the DP2/CRTH2 receptors present in inflammatory cells such as Th2, eosinophils and basophils. The anti-inflammatory action of PGD₂ is associated with the presence of DP1 receptors in DCs and prevents them from migrating from the lungs to lymph nodes and may play a role in the immune adaptive response to antigens by reducing the proliferation of cytokines by antigen- specific T cells. The PGD₂ is not excreted in the urine in its initial form. The first and most abundant metabolite of PGD₂ is 11β-PGF_{2α}, that also has biological activity such as bronchoconstriction and contraction of the coronary arteries and is synthesized enzymatically by the action of NADPH-dependent 11-ketoreductase enzyme and also [64-66].

Oxidants/Antioxidants Imbalance in Asthma

Antioxidants protect cells and tissues from the continuous production of ROS and RNS from normal organism metabolism [67]. An imbalance in the state of redox homeostasis of the airways is a determining factor in the initiation of asthma and its severity [11]. Consequently, asthma is characterized by a loss of both enzymatic and non-enzymatic antioxidant activity as demonstrated by Sackesen et al. (2008) [68] where they found that GPx and SOD enzymes as well as non-enzymatic compounds including glutathione, ascorbic acid, α -tocopherol, lycopene and β -carotene were found in significantly lower concentrations in asthmatic children. The loss of antioxidant enzymatic activity such as SOD, CAT and GPx, reflects the oxidative stress in the airways of asthmatic patients [37].

Genetic Component of The Oxidative Stress in Asthma

The genetic component of asthma is also reflected in the oxidative stress. Several GSTs are genetically expressed in the lungs. Different polymorphisms, with a high frequency in the population, lead to a loss in the expression of these enzymes, reflecting a pathophysiological alteration that results in an inflammation of the respiratory tract and in a consequent increase in respiratory symptoms when the organism is exposed to ozone and is associated with an increase of IgE and a histamine response. The same effect occurs in the case of SOD in which a genetic mutation leads to a change in the secondary structure of the protein which affects the targeting of the protein to the mitochondria [11].

Clinical Monitoring of Asthma and Prospects

Oxidant species are, to a certain degree, increased in asthmatic patients. The eosinophil peroxidase and myeloperoxidase are increased in the blood, induced sputum and BAL fluid of these subjects [11]. Various compounds related to oxidative stress have been found in the various biological fluids like urine, exhaled air, plasma, saliva and BAL fluid of patients with asthma. Generally, these compounds have a positive correlation with the severity of this disease, including malondialdehyde resulting from lipid peroxidation [69], thiobarbituric acid reactive substances [70], glutathione disulfide [71], H_2O_2 [70], NO [72], isoprostanes [73] and ethane [74].

Current therapy with anti-inflammatory corticosteroids and β_2 -agonists remains as a standard treatment. Consequently, the activity or the number of cells involved in the production of ROS and RNS is decreased, thus avoiding oxidative stress [11]. Several efforts have been made in the treatment with antioxidants such as certain precursors of thiols (organosulfur compounds; e.g. N-acetyl cysteine) capable of reducing airway inflammation and hyperreactivity [75], vitamins (e.g. vitamin A, E and C) that protect against the action of both endogenous and exogenous reactive species and polyphenols (e.g. quercetin and epigallocatechin-3-gallate) which, in addition to their antioxidant activity, also stabilize mast cells [76], molecules that mimic antioxidant enzymes and nanomaterials such as fullerenes that have the ability to capture radical species due to their ability to absorb electrons [77].

Some studies relate dietary fatty acids to inflammation. A diet rich in saturated fatty acids can induce the prostaglandin production such as PGE2 and COX-2 expression leading to muscle inflammation. In the other hand, a diet rich in unsaturated fatty acids shows a tendency to decrease the expression of COX-2 by saturated fatty acids [78].

Metabolomics

The future of asthma therapy involves a better understanding of the mechanisms of oxidative stress in the body. Metabolomics is an 'omic' science dedicated to the study of small molecules with less than 1000 Da such as volatile organic compounds, peptides, amino acids, nucleic acids, carbohydrates, organic acids, vitamins and polyphenols. An example of a metabolomic science is the volatome. It dedicates to the study of volatile organic metabolites (VOMs), a very diverse group of compounds whose classification is based on their retention time in chromatography and boiling point that varies between 50 and 260 °C. These compounds are present in several matrices of the body such as urine, blood, feces and sweat.

If there are indications of dogs that discriminated patients with certain pathologies such as cancer from different matrices such as urine, skin, exhaled air and blood [79, 80], it is logical to think that sensitive techniques, such as chromatography (e.g. ultra high performance liquid chromatography - UHPLC and gas chromatography - GC), can also do the same discrimination.

Since the metabolites represent the expression of the genome, transcriptome and proteome, they may represent the phenotype of an individual at any given time [81]. Thus, it is possible that the metabolic (i.e. volatome) profile of a person with a certain disease, even in an early stage, is to a certain degree altered when compared to a healthy person, and it is also expected that different diseases will also have different metabolic patterns or 'biosignatures'. A simple approach is the measurement of blood sugar levels on diabetes that help to control the insulin levels [81, 82]. The volatile 'biosignatures' have been correlated with diseases such as cancer [83], cystic fibrosis [84], diabetes [85] and tuberculosis [86]. In the same way, certain chemical tests with a metabolomic concept have been carried out to evaluate the inflammation of the airways of asthmatic patients. Some biochemical markers include exhaled NO fraction, exhaled carbon monoxide, pH of the exhaled breath condensate and leukotriene quantification [87-89]. However, these tests, which are also used clinically, are only restricted to an inflammation-related biomarker where it is observed a significant inter- and intra-individual variation [82]. The 'biosignatures' obtained through the various studies may lead to the development of devices or techniques that allows the early detection of diseases resulting in more efficient treatments, reduction of suffering by patients and consequently a reduction in mortality [81].

Being asthma a chronic disease, it is believed that its metabolic profile is in some way altered. In this context, several studies have been carried out in order to establish a metabolic signature for asthma. In two studies carried out by Caldeira et al. [90, 91], the volatome profile of the exhaled air was traced in children with asthma. In the first study, conducted in 2011, they

compared the volatometric profile of a group of pediatric asthmatic patients (n = 33) with the profile of a group of pediatric individuals without any signs of asthma (control group; n = 15). In this study, they identified 28 VOMs with discriminatory power between the asthmatic group and the control group. In the second study, carried out in 2012, they compared the profile of a group of asthmatic patients in pediatric age (n = 32) with a control group (n = 27) and identified 6 alkanes VOMs characteristic of the asthmatic population. In 2011, Saude et al. [92] differentiated the urine metabolic profile of 135 children: 73 with controlled asthma, 20 with uncontrolled asthma and 42 as a control group. In 2015, Adamko et al. [93] distinguished the metabolic profiles of asthmatic patients (n = 133) from COPD patients (n = 38) on the uncontrolled and controlled forms (n = 54 and n = 23 respectively).

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