

# Oxidants and asthma

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Many decades of research have produced a significant amount of data showing increased oxidative stress in asthma and indicating a potential role for oxidants in the pathogenesis of the disease, particularly during exacerbations. Putatively relevant pro-oxidative mechanisms have also been identified. Currently available asthma drugs are generally effective for the treatment of the disease, but their effects on oxidative stress have still not been completely elucidated. From the data available in the literature one can conclude that antioxidant compounds may have a potential role in the treatment of asthma, especially of asthma exacerbations. More convincing evidence from controlled clinical trials is required.

hydrogen peroxide ( $H_2O_2$ ), either spontaneously or under the influence of superoxide dismutases (SODs). Although  $O_2^-$  and  $H_2O_2$  themselves are moderate oxidants, both species are critical for the formation of potent cytotoxic radicals in biological systems through their interaction with other molecules. For instance, the lysosomal enzymes myeloperoxidase (MPO) from neutrophils and monocytes/macrophages and the eosinophil peroxidase (EPO) catalyse the oxidation of halides ( $Cl^-$ ,  $Br^-$ , and  $I^-$ ) by  $H_2O_2$  to form hypohalous acids (HOCl or HOBr). MPO produces predominantly hypochlorous acid (HOCl) whereas EPO produces more hypobromous acid (HOBr). Hypohalous acid production is important in the host defence against infectious agents, but during this reaction the hydroxyl radical ( $OH^-$ ) is also produced, which is a powerful and indiscriminate oxidant.

Oxidant generation is part of the normal metabolism of many types of cells and is critical for cell homeostasis. To protect itself against exposure to noxious oxidants, the lung has a well developed antioxidant system.<sup>1–2</sup> When an imbalance occurs between oxidants and antioxidants in favour of oxidants, oxidative stress is said to occur. Many experimental and clinical data suggest that oxidants play a role in the pathogenesis of several respiratory disorders, including bronchial asthma.

In particular, there is increasing evidence that the chronic airway inflammation typical of asthma results in an increased oxidative stress to the airways. Also, many of the triggers for asthma exacerbations, including viral infections and air pollutants, may activate the production of oxidants, triggering increased inflammation which produces asthmatic symptoms.

Eosinophils possess several times greater capacity for generating oxidants than neutrophils, and the EPO content of eosinophils is several times higher than that of MPO in neutrophils.<sup>3–9</sup> MPO and EPO derived reactive oxygen species can also interact with nitrite ( $NO_2^-$ ) and  $H_2O_2$  leading to the formation of reactive nitrogen species (RNS; nitrosants). A powerful oxidant, the radical peroxynitrite ( $ONOO^-$ ), is produced from the reaction between  $O_2^-$  and nitric oxide (NO) which is increased in the asthmatic airways.<sup>3–8 10</sup>

As in many other inflammatory conditions, the oxidative “burst” in asthma is a non-specific process initiated by the concurrent action of numerous inflammatory pathways. Indeed, several asthma mediators including lipid mediators, chemokines, adhesion molecules, and eosinophil granule proteins are potential promoters of oxidant production.<sup>4</sup>

## OXIDANTS AND INFLAMMATION IN ASTHMA

### Sources

The inflammatory cells recruited to the asthmatic airways have an exceptional capacity for producing oxidants. Once recruited in the airspaces, inflammatory cells may become activated and generate reactive oxidants in response to various stimuli. Activated eosinophils, neutrophils, monocytes, and macrophages, and also resident cells such as bronchial epithelial cells, can generate oxidants.<sup>3–8</sup> The univalent reaction of oxygen to superoxide anion ( $O_2^-$ ) is an important step in the formation of oxidants. Sources of superoxide anion include primarily the membrane associated NADPH oxidase dependent complex, the cytosolic xanthine oxidase system, and the mitochondrial respiration chain (fig 1).<sup>3–8</sup> Superoxide anion is then converted to

### Effects of oxidants in experimental settings

In experimental models, oxidants can produce many of the features typical of asthma. They induce bronchoconstriction and increase airway responsiveness to several agonists.<sup>3–8</sup> The release into the airways of tachykinins and neurokinins and the decrease of  $\beta_2$  adrenergic receptor, cholinesterase, and neutral endopeptidase activities have been documented in these experimental conditions.<sup>3–8</sup> Oxidants can also lead to increased permeability of the airways in these models.<sup>3–8</sup> Conversely, reducing agents exert a relaxing effect on airway smooth muscle and can inhibit bronchial smooth muscle contraction and prevent airway hyperresponsiveness in several experimental models.<sup>3–8</sup>

Experimental exposure to oxidants induces many degrees of injury to bronchial epithelial cells until cell death. EPO can induce lysis of

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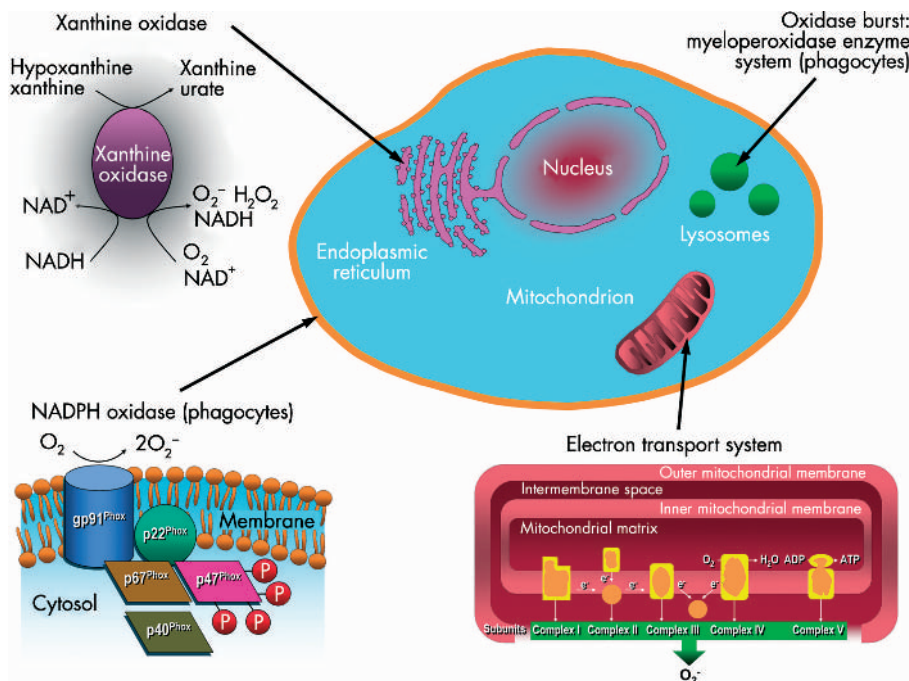


Figure 1 Main cell sources of oxidants.

bronchial epithelial cells at concentrations that have been found in the asthmatic airways.<sup>3-8</sup> Oxidants induce increased apoptosis of bronchial epithelial cells from asthmatic subjects.<sup>11-12</sup>

In vitro exposure of structural and inflammatory cells of the lungs to oxidants induces the release of pro-inflammatory mediators including cytokines, chemokines (and their receptors), growth factors, arachidonic acid metabolites, and adhesion molecules (and their ligands) involved in inflammatory cell recruitment in asthma.<sup>3-8</sup>

Within the nucleus, oxidants induce changes in gene expression. Interestingly, in vitro oxidative stress can induce activation of nuclear factor  $\kappa$ B (NF- $\kappa$ B) and activator protein 1 (AP-1), two pivotal regulators of inflammatory processes.<sup>13</sup> Both these transcription factors are activated in the bronchial epithelial cells of asthmatic subjects.<sup>14</sup> Such activation can potentially explain the increased expression of many pro-inflammatory mediators found in the airways of asthmatic patients.<sup>14</sup>

## OXIDANTS IN ASTHMA

Many studies suggest that levels of oxidative stress are increased in children and in adults with asthma, not only in their lungs but also in the circulation.

Excessive production of oxidants occurs spontaneously or after stimulation in blood leucocytes of stable asthmatics compared with normal subjects.<sup>3-8, 15</sup> Levels of EPO and/or MPO are increased in the peripheral blood, induced sputum, and bronchoalveolar lavage (BAL) fluid from patients with stable asthma.<sup>3-9, 16</sup>

Recently, increased levels of many direct and indirect markers of oxidative stress (including malondialdehyde, thiobarbituric acid reactive products (TBARs), and  $H_2O_2$ ) have been found in the urine, plasma, sputum, BAL fluid, and lung tissues of patients with asthma.<sup>3-8</sup> Their level is often related to the severity of the disease and is inversely related to the degree of stability.<sup>3-8</sup> Furthermore, analysis of exhaled breath and exhaled condensate has recently allowed direct assessment of  $H_2O_2$  and nitric oxide (NO) and measurement of several indirect byproducts of oxidation in those samples.<sup>17, 18</sup> The latter are footprints of oxidation on

different substrates such as proteins (nitrotyrosine), lipids (isoprostanes and ethane), and DNA (hydroxydeoxyguanosine).<sup>17, 18</sup> There is a lot of interest in the effect of these new non-invasive markers on oxidative stress and in their possible clinical application<sup>17-19</sup> as their concentrations are significantly increased in asthma.

The expression of nitrotyrosine is increased also in bronchial and bronchiolar epithelial cells, in smooth muscle cells, and in eosinophils of bronchial airways and lung parenchyma of patients with stable asthma.<sup>20-22</sup> Excessive production of 3-bromotyrosine, another marker of oxidative stress, has been reported in the airways of patients with severe asthma.<sup>23</sup> A significant increase in 3-bromotyrosine has also been described in the proteins of sputum of asthmatic subjects. This finding is of particular interest as the level of 3-bromotyrosine is strongly related to levels of EPO.<sup>9, 24</sup> However, whether peroxidases simply reflect granulocytic inflammation or whether they actively contribute to tissue damage in asthma remains to be determined.

Taken together, these data indicate that oxidative stress is enhanced in asthma, and that such redox imbalance is not confined within the lungs.

## ANTIOXIDANT DEFENCES IN ASTHMA

Many controlled studies suggest that there is a deficiency of antioxidants with few studies indicating an activation of the pathways protecting lung cells from oxidant mediated damage in the lungs or circulation of asthmatic subjects.<sup>3-8, 15</sup> For instance, a marked reduction in plasma antioxidant capacity occurs during exacerbations.<sup>8</sup> In stable conditions, CuZn-SOD activity is lower in steroid naive asthmatics than in normal subjects.<sup>25</sup> Similarly, peroxynitrite inhibitory activity, an antioxidant system, is reduced in the sputum of patients with stable asthma and its level is positively related to airway responsiveness and negatively related to forced expiratory volume in 1 second (FEV<sub>1</sub>) and the degree of sputum eosinophilia.<sup>26</sup>

Free NO levels are generally low in the respiratory system as the NO produced by endogenous NO synthases (NOSS) is complexed with reducing agents like thiols, including glutathione, to form a major reservoir of NO related

bioactivity in the form of S-nitrosothiols (SNOs) which are relatively resistant to oxidants.<sup>10</sup> Interestingly, airway levels of the endogenous bronchodilator S-nitrosoglutathione (GSNO) are reduced in children with near fatal asthma.<sup>27</sup> It has been hypothesised that GSNO could be broken down by oxidants in the asthmatic lung and that its catabolism could have an inhibitory effect on airway smooth muscle relaxation.<sup>28</sup>

Among antioxidant systems, cyclin dependent kinase inhibitor p21<sup>CIP1/WAF1</sup>, a protein that protects against oxidative stress, and extracellular glutathione peroxidase are increased in bronchial epithelial cells of asthmatic patients.<sup>29-30</sup> A link between asthma and selenium deficiency (glutathione peroxidase is one of many selenium dependent antioxidant enzymes) has also been hypothesised.<sup>31</sup> However, a controlled trial did not show any significant benefit of short term selenium supplementation in patients with intrinsic asthma.<sup>32</sup>

Vitamin C has also been studied for its possible inverse relationship with increased risk of asthma<sup>33</sup> but, to date, evidence from randomised controlled trials is insufficient to recommend a specific role for vitamin C in the treatment of asthma.<sup>34</sup> The antioxidant vitamin E inhibits IgE responses to allergic stimuli in animals and dietary vitamin E levels are inversely related to the incidence of asthma.<sup>34</sup> Recent studies found that supplementation with vitamin C and vitamin E reduced ozone related decrement in lung function in asthmatic subjects, particularly in those with genetically determined increased susceptibility to oxidant stress.<sup>35-36</sup>

Many promising new antioxidants such as nitrones (spin trap antioxidants that inhibit the formation of intracellular oxidants by forming stable compounds) and non-peptidyl SOD analogues<sup>37-38</sup> are now ready to enter clinical trials.

## EFFECT OF ASTHMA DRUGS ON THE OXIDANT/ANTIOXIDANT BALANCE

Although glucocorticoids do not inhibit in vitro oxidant production from eosinophils,<sup>39</sup> inhaled glucocorticoids decrease levels of H<sub>2</sub>O<sub>2</sub> in exhaled breath condensate of asthmatics.<sup>17-18</sup> Similarly, treatment with low doses of inhaled glucocorticoids decreases total nitrate and nitrite concentrations both in exhaled breath condensate and in sputum of patients with stable asthma.<sup>40</sup> Treatment with inhaled glucocorticoids also normalises the reduced bronchial epithelial CuZn-SOD specific activity.<sup>25</sup> It is unknown whether the efficacy of glucocorticoid treatment in asthma is somehow linked to their influence on oxidant/antioxidant balance.

In vitro, short acting and long acting inhaled  $\beta_2$  agonists have an antioxidant effect on neutrophils and eosinophils. However, to achieve their antioxidant effect, supratherapeutic concentrations are required and therefore the clinical relevance of such an effect is unclear. It has been hypothesised that the antioxidant activity of  $\beta_2$  agonists is related to the number of hydroxyl groups on the phenol rings within their molecular structure.<sup>41-42</sup>

Aminophylline reduces the generation of oxidants in an animal model of endotoxin induced bronchial hyperresponsiveness. Theophylline also inhibits platelet activating factor (PAF) induced and interleukin (IL)-5 induced oxidant release from blood eosinophils in a dose dependent manner, indicating that theophylline at high concentrations could have a significant antioxidant effect.<sup>43-44</sup>

## EXACERBATIONS

Most of the studies on oxidative stress in asthma have focused on the oxidant/antioxidant imbalance that occurs in stable asthma. The superimposed effects of exacerbations have received much less attention.

Episodic worsening of asthma is associated with increased airway inflammation.<sup>4-45</sup> There is also evidence of enhanced oxidative stress during exacerbations, both systemically and locally. However, a direct correlation between increased oxidative burden and changes in pulmonary function and/or airway inflammation described during exacerbations remains speculative.<sup>8</sup> In this context, a hypothesis that links exacerbations of asthma to dietary antioxidant deficiency has been proposed.<sup>8-32</sup> Furthermore, several indirect markers of oxidative stress such as H<sub>2</sub>O<sub>2</sub> and isoprostanes are increased in exhaled air, sputum, and BAL fluid during exacerbations and after allergen exposure.<sup>8-17-18</sup>

Respiratory viruses represent the most important causes of asthma exacerbations. Rhinoviruses are the virus type most frequently identified in respiratory tract specimens during exacerbations of asthma, both in children and in adults.<sup>12</sup> Experimental rhinovirus infection of asthmatic patients can induce an inflammatory response in the airways associated with variable airflow obstruction and increased airway hyperresponsiveness.<sup>12</sup> Rhinovirus induced airway inflammatory responses involve eosinophils and neutrophils, possibly recruited via cytokines or chemokines released by bronchial epithelial cells or T cells.<sup>46</sup> Rhinovirus infection of respiratory epithelial cells causes intracellular oxidant generation which is a crucial step in the activation of NF- $\kappa$ B and in the following production of proinflammatory adhesion molecules and cytokines.<sup>47</sup> Reducing agents inhibit both rhinovirus induced oxidant generation and inflammatory mediator production and release.<sup>47-48</sup>

Taken together, these observations provide evidence of an increased oxidative burden in asthma exacerbations. However, until now, most of this evidence has been indirect and the pathogenetic mechanisms putatively involved have been investigated mostly in vitro.

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