Oxidative Stress in the Lung Tissue – Sources of Reactive Oxygen Species and Antioxidant Defence

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Abstract: Reactive oxygen species are oxygen-based molecules readily reacting with various compounds. It is already known that they play significant role in many physiological as well as pathological body processes. The aim of our review is to briefly summarize our knowledge of possible ROS sources in the lung tissue.

Introduction

There is no doubt that the so-called reactive oxygen species (ROS) play a significant role in living organisms. For a long time it has been speculated that ROS are involved in the pathogenesis of many diseases and pathologic processes. However, only recently there is mounting evidence that ROS play an important part in the complex physiological processes such as cell signalling, apoptosis, etc. One of the organs commonly affected by ROS generation is the lungs. It is obvious that, having a large surface that is constantly in contact with air oxygen and pollutants, lungs is a site of major ROS production. This has lead to the evolution of an antioxidant defence system to protect the lungs from substantial damage. When the fragile balance between ROS production and the defensive capacity of the antioxidant system is violated, pathological reactions may cause injury or disease. In this review we discuss the possible sources of ROS in the lungs and the main components of antioxidant defence.

Biochemistry of ROS

ROS are oxygen-containing molecules that are capable of either accepting or donating a free electron, thus they are, to some extent, unstable and react with other molecules. This reaction may lead to the generation of other, sometimes even more reactive molecules. The first step in the complex chain of ROS is a one-electron reduction of molecular oxygen, leading to production of superoxide O_2^-. O_2^- is unstable and quickly undergoes another reduction to hydrogen peroxide H_2O_2, either spontaneously or in a much faster reaction catalysed by superoxide dismutase (SOD). H_2O_2 is relatively stable and can migrate from its site of origin; therefore it is capable of affecting a large scale of important cellular molecules. It can also turn into highly reactive hydroxyl radical $OH^\bullet$ (Fenton's reaction, catalysed by free iron).

Cellular injury caused by ROS is associated with their impact on cellular structure (membrane lipoperoxidation, DNA strand breaks) and function (changes in enzymatic activity, signalling). The effect of ROS depends on their concentration – while structural changes need relatively high ROS concentrations (DNA strand breaks were seen with H_2O_2 concentrations between 20–120 mol/L [1]), lower levels of ROS may modulate cellular processes involved in different types of injury such as proliferation, apoptosis and necrosis, that are controlled by so called "redox regulation" at the transcriptional level. The evidence that ROS regulate transcription factors NF-kB [2] and activation protein 1 and p53 through the modulation of cellular redox state is already convincing [3]. An interesting example

is the possible activation of lung mast cells by ROS at the onset of chronic hypoxia leading to increased production of metalloproteases and specific cleavage of collagen, which in turn triggers remodelling of pulmonary vessels [4].

ROS sources on a subcellular level

Virtually every cell in human body has an apparatus necessary for the generation of reactive oxygen intermediates and is therefore capable of producing ROS in various amounts.

1) One of the main ROS sources under physiologic conditions is mitochondria. In the inner membrane they contain an electron chain transfer system for ATP generation, permanently generating O_2^- (it is estimated that some 1–2% or even 4% of O_2 consumption undergoes transformation to O_2^- [5, 6]. The outer mitochondrial membrane carries monoamine oxidase, a heme-containing enzyme catalysing oxidative deamination of organic amines, producing large amounts of H_2O_2 in the mitochondrial matrix as well as in the cytosol directly.
2) Another quantitatively important system generating O_2^- is NADPH oxidase, originally found in neutrophils (Nox). It is formed from 5 subunits, the most important of them being gp91 bound to the cellular membrane. Upon stimulation, the cytoplasmic subunits link together and migrate towards the membrane gp91, thus activating superoxide production [7]. Recently it has been shown that many other cellular types (such as endothelia or smooth muscle cells) possess a similar superoxide-generating system, called NADPH-like oxidase, activated by various hormones and cytokines. 1) NADPH-like oxidase is permanently in a fully preassembled state and constantly producing low amounts of O_2^-, most likely with a regulatory function [8]; 2) upon stimulation, its peak production is much lower compared to Nox and is significantly delayed, nonetheless playing important role in some pathologic states such as ischemia-reperfusion, hypertension or atherosclerosis [9] 3) its superoxide production is directed mainly intracellularly as opposed to NADPHoxidase-generated superoxide in neutrophils, where it serves as a defence mechanism outside the cell.
3) Some cells contain xanthine oxidoreductase (XOR), which catalyses the transformation of hypoxanthine to xanthine and xanthine to uric acid. Under physiological conditions, the XOR is present in the form of xanthine dehydrogenase (XD). XD can be modified, either reversibly (by oxidation of sulfhydryls) or irreversibly (by mild proteolysis) to xanthine oxidase (XO), generating large amounts of H_2O_2 as well as O_2^-. It is speculated that this process gains importance especially after ischemia-reoxygenation, when the XD-XO transformation is rapid and hypoxic tissue contains vast amounts of substrate for XO. However, latest research suggests that the XD-XO conversion may not be of clinical significance and that total XOR activity is

important, even XD being able to generate ROS [10]. Recently it has been discovered that XOR can transform nitrates and nitrites to nitrites and NO, respectively (even in anoxia, in contrast to NOS). The enzyme itself is able to catalyse the reaction of NO with O_2^-, thus generating highly reactive peroxynitrite as well [11]. On the other hand, it has to be noted that XOR is a source of uric acid, which is known to be potent oxidant scavenger.

4) ROS can be generated as by-products during metabolism of arachidonic acid, which to some degree takes place in practically every cell. Enzymes participating in the process are cyclooxygenase, lipooxygenase and cytochrome P-450 [12]. Arachidonic acid may be a source of ROS even by a non-enzymatic process.
5) Practically all cells possess P450 cytochrome oxidase, a heme-containing enzyme system localised in mitochondria or microsomes. It is a superfamily of isoenzymes that mostly function as monooxygenase, but it can catalyse an intramolecular transfer of oxygen as well. As our knowledge grew with time, it became clear that P450 isoenzymes participate in the metabolism of steroid hormones, cholesterol and its catabolism to bile acids, arachidonic acid and eicosanoids [13]. It catalyses the hydroxylation of vitamin D_3 and retinoic acid, and plays an important role in the metabolism of many xenobiotics. The underlying concept of its activity is a multi step transfer of 2 electrons to a substrate while binding one oxygen atom to it, the second being reduced to water. Part of the oxygen involved is inevitably reduced to superoxide.
6) Lysosomal membrane contains an electron transport system, which helps ensure optimal intralysosomal pH by pumping protons. This system promotes a three-electron reduction of oxygen, thus leading to generation of highly reactive $OH^{\bullet}$.
7) Myeloperoxidase is a heme containing enzyme present in neutrophils and eosinophils, where it catalyses the reaction of H_2O_2 with various substrates leading to generation of potent oxidants such as hypochlorous acid [14].

Possible cellular sources of ROS in lungs

Neutrophils have been known to produce ROS as a part of their bactericidal activity. In the cytoplasmic membrane they contain NADPH oxidase, which generates large amounts of O_2^-. Its activation is responsible for the „respiratory burst." Another enzyme contributing to the complex defence mechanism is myeloperoxidase, which catalyzes the reaction of H_2O_2 with halide anions upon neutrophil stimulation [15]. A chloride anion is by far the most frequent one, therefore MPO produces mostly hypochlorous acid $HOCl^-$, which is highly reactive [16]. Xanthine oxidoreductase has been detected in neutrophils as well.

However, neutrophils present only minor cellular type in lungs under physiologic conditions and it is only after stimulation during inflammation that they migrate into pulmonary circulation in vast amounts.

Eosinophils are also a potent cellular source of ROS, especially in some allergic or infectious diseases [17]. Similar to neutrophils, their membrane contains NADPH oxidase which generates superoxide. Cytoplasmic peroxidase (eosinophilic peroxidase, analogue of myeloperoxidase in neutrophils) contributes significantly to bactericidal activity or damaging effects of eosinophils, producing mostly hypochlorous acid (HOCl).

Alveolar macrophages are phagocyting cells present in the lungs in large numbers and therefore form the first line of lung defence against infection. Similar to neutrophils, their main source of ROS is the membrane NADPH oxidase, generating O_2^- [18]. It was suggested that alveolar macrophages are the major source of ROS under physiologic conditions [6].

Peripheral monocytes-macrophages attracted by inflammatory cytokines contribute substantially to the damage in pulmonary diseases – recently it has been shown that after stimulation and differentiation into macrophages these cells are capable of producing superoxide by XOR [19] which plays a significant role in acute lung injury (as opposed to invading neutrophils, where XOR is silent).

Mast cells are also present in lungs, but so far the reports about their ROS production are rather confusing. However, there is certain data proving that they might be able to generate ROS [20].

Type II pneumocytes form a part of alveolar epithelium. These cuboidal cells with very active metabolism produce surfactant and are thought to function as precursor cells for type I pneumocytes in the case of increased destruction of alveolar epithelium. Recent studies show that even type II epithelial cells possess enzymatic properties for production of some ROS [21, 22].

Endothelial cells, thanks to rich lung vascularization, present another substantial cellular mass. In recent years there has been growing evidence that even these cells can present a source of ROS and participate in oxidative stress and lung injury under pathological conditions. They contain xanthine oxidoreductase complex which seems to become stimulated mostly after hypoxia [23]. Moreover, it has been shown that membrane-bound NADPH oxidase is also present, with some differences from the phagocytic type [24, 25]. Endothelial cells are capable of releasing substantial amounts of O_2^- into the extracellular space, possibly via membrane anion channels [26]. Formation of another ROS, highly reactive OH, can be catalyzed by iron ions present in proximity of the endothelial surface [26].

Smooth muscle cells (either in airway or in vessel walls) may act as another ROS source as it has been shown that their membrane contains NADPH-like oxidase generating O_2^- [27, 28]. ROS produced by airway smooth muscle cells play a significant role in airway hyperreactivity.

Lung fibroblasts have also been proved to produce ROS, especially after stimulation by inflammatory cytokines. Thannickal [29] reports presence of two different systems, both membrane-bound. The first is NADPH oxidase

(phagocyte-like) which generates O_2^- intracellularly. The second enzyme is NADH oxidase, which produces H_2O_2 directly into the extracellular space [30].

It is evident that cells of practically every type present in the lungs are capable of producing some ROS. The role of different cell types in the pathogenesis of specific diseases is far from being clear. While it is believed that during inflammation phagocytes are the main source of the oxidative stress, the role of different cell types in a variety of other conditions is still to be discovered. This deficit in our knowledge is partly due to lack of appropriate methods of localization of the ROS formation in the lung tissue in situ. Yet even more important seems to be the role of ROS in various physiological processes such as signalling or apoptosis.

Antioxidant defence

Practically every cell is endowed with mechanisms protecting it against the damaging effects of ROS. We recognize antioxidants both intra- and extracellular and we can divide them into enzymatic and non-enzymatic categories as well. Gutteridge and Halliwell [31] classified antioxidants as primary (preventing oxidant formation), secondary (scavenging ROS) and tertiary (removing or repairing oxidatively modified molecules) which may be constitutive, inducible or dietary according to their origin.

The lung is directly exposed to the environment and to oxygen at higher partial pressure than other organs; its antioxidant defence is, therefore, particularly important [32]. First in the line of enzymatic ROS degradation is superoxide dismutase (SOD). This enzyme exists in 3 forms – a) Cu/Zn SOD present mainly in cytosolic matrix, b) MnSOD localized preferentially in mitochondria and c) extracellular SOD. All SODs efficiently catalyze transformation of O_2^- into H_2O_2, yet its role in the antioxidant defense is not clear. Adding SOD was reported to enhance as well as to limit lipid peroxidation and membrane damage [33] which may result from different local concentrations of Fe+ and/or different concentration of enzymes protecting the cells against hydrogen peroxide. It is also clear that cells contain another enzyme protecting them against hydrogen peroxide. This function is fulfilled by the catalase and glutathione system. Catalase is localized intracellularly, especially in peroxisomes, and works efficiently under high concentrations of H_2O_2. In low concentrations it is mainly the reduced form of glutathione (GSH) that transforms hydrogen peroxide [34, 35]. The ability of the glutathione system to reduce H_2O_2 depends on the ratio between GSH and the oxidized form (GSSG), which in turn depends on two processes: transport of GSSG out of the cell and the capacity of GSH reductase – an enzyme which converts GSSG to GSH. The GSH concentration in the alveolar lavage fluid exceeds its plasmatic concentration by more than 100 fold [35] illustrating high capacity of this system in the lung. Regeneration of GSH requires reduced nicotinamid adenine dinucleotide phosphate (NADPH) that is supplied through

glucoso-6-phosphate dehydrogenase (G6PD) activity in the hexoso monophospate shunt. Moreover, there are other enzymatic systems that may contribute, directly or indirectly, to defence against oxidative stress. Among these are thioredoxins, their protective effect lies in the reduction of disulfidic bonds.

The non-enzymatic antioxidants are mostly "scavengers" of free radicals, such as vitamin C, vitamin E (inhibits oxidation of membrane lipids), uric acid (efficient scavenger of peroxynitrite, present in plasma and airway lining fluid), albumin, bilirubin, glutathione [36] or N-acetylcysteine (NAC). NAC is a potent drug which acts directly by reacting with ROS (forming NAC disulfides in the end) and indirectly, serving as a GSH precursor [37]. It does not prevent neutrophil influx to lungs, but prevents their oxidative burst [38]. However, as with many antioxidant substances, NAC in high doses can exhibit prooxidative effects [39].
Non-enzymatic antioxidant could also prevent ROS formation like allopurinol which proved to be a potent inhibitor of xanthine oxidoreductase.

As the knowledge about ROS and their role in many pathologic processes grows, it is logical that many natural as well as synthetic substances (some of them mentioned above) are being tested in search of new therapeutic approaches utilizing their antioxidant properties. Among the most frequently investigated experimentally were glutathione [40], vitamins C [41, 42] and E [43], N-acetylcysteine [38, 44], melatonin [45] or allopurinol in model of acute lung injury [19]. A lot of researchers focused on enhancing enzymatic defence by administering SOD or catalase either intravenously [46] or intratracheally [47].

Some of the agents were already used in small clinical trials on human patients with promising results (glutathione [48, 49, 50]; vitamin C [51]; SOD [52]; NAC [53]). Yet, so far none of them have become routinely used in clinical practice as there are still some confusing points to be resolved.

Summary

There is little doubt that ROS play an important role in pathogenesis of pulmonary diseases as well as many regulatory physiologic functions. Their importance depends on the subtle balance between ROS production and the defensive capacity of antioxidant systems. In addition, the location of their production also plays an important role. Many cells of the lung pulmonary tissue have the capacity to form ROS, however the real ROS sources in different pathological processes in the lung are far from being clear.

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