Eur Respir J, 1993, 6, 334–336
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

EDITORIAL

How the lung deals with oxidants

K.L. Maier*

Oxidants are compounds capable of withdrawing electrons from target atoms or molecules, thus initiating chemical reactions. In biological systems, oxidative modifications are often related to structural, functional and regulatory changes. Partially reduced oxygen species, also known as oxygen-derived free radicals, represent the most important group of biologically relevant oxidants. Significant amounts of oxygen-derived free radicals are produced by inflammatory cells, capable of destroying neoplastic tissue, altering cellular function, modulating inflammation and exhibiting antimicrobial defence.

In the lung, reactive oxygen metabolites mainly originate from alveolar macrophages and, during inflammation, also from invading neutrophils and eosinophils. Phagocytic cells are endowed with a complex enzyme system located in the plasma membrane, the nicotinamide-adenine-dinucleotide phosphate (reduced form) (NADPH) (or burst) oxidase [1], which is dormant in silent cells, but activated in stimulated cells to catalyse the reduction of molecular oxygen to the superoxide anion (O_2^-). In a subsequent step, O_2^- dismutates to hydrogen peroxide (H_2O_2) and molecular oxygen, catalysed by the enzyme superoxide dismutase. In the presence of metal ions, highly reactive hydroxyl radicals (OH^-) arise from O_2^- and H_2O_2 *via* the Haber-Weiss or Fenton reaction, respectively [2]. Hydroxyl radicals are also formed by neutrophils and monocytes from H_2O_2 in a myeloperoxidase-dependent reaction [3]. However, the main function of myeloperoxidase from neutrophils (and chloroperoxidase from eosinophils) is the formation of cytotoxic hypochlorous acid (HOCl), a process which also consumes H_2O_2. The end-point of this pathway is the chlorination of primary or secondary amines to cytotoxic N-chloramines ((R_1R_2)N-Cl), which are also potent oxidants. The myeloperoxidase is an inflammatory marker, which substantially influences the composition of the oxidant pattern in the lung.

Production of oxygen-derived free radicals by the burst-oxidase seems not to be restricted to phagocytic cells. Recent studies have revealed that human fibroblasts possess an NADPH oxidase system exhibiting strong similarities to the phagocytic NADPH oxidase [4]. Moreover, alveolar type II cells have been shown to release oxygen-derived free radicals in a similar amount to alveolar macrophages upon treatment with various stimulants [5], suggesting the existence of a NADPH oxidase in these cells. Genetic deficiency of NADPH oxidase activity (as known to exist in chronic granulomatous disease) makes the individuals highly susceptible to pathogens. This observation underlines the outstanding role of oxygen-derived free radicals as part of the immunological defence machinery in the lung.

Apart from the beneficial microbial effect of reactive oxygen metabolites, current discussion suggests that they participate in pathophysiological mechanisms of lung parenchymal injury [6]. Accumulation of inflammatory cells, such as neutrophils or eosinophils, in the airways results in an exaggerated release of reactive oxygen metabolites, representing a potential risk of lung tissue damage. Data have accumulated indicating an increased oxidant burden of neutrophilic origin in a number of lung diseases, such as the adult respiratory distress syndrome [7], idiopathic pulmonary fibrosis [8], bronchitis [9, 10], cystic fibrosis [11], and immune complex injury [12]. The role of oxidants in the initial stage of lung diseases is still unclear, but it is obvious that reactive oxygen metabolites must be removed rapidly from the organ, before they can cause cellular dysfunction or cell death resulting in impaired lung functions.

The nature of injury depends on the kind and reactivity of the oxidant acting in the epithelial lining fluid (ELF) or lung tissue. Hydrogen peroxide reacts rather slowly with sulphur-containing amino acids, and the small rate constants for these reactions suggests that it may not be significant at the very low H_2O_2 concentrations likely to be present *in vivo*. In contrast, HOCl and (R_1R_2)N-Cl readily attack methionine and cysteine residues, and rapidly inactivate the α_1-proteinase inhibitor already at low concentrations in the range of 1–10 μ M, oxidant concentrations which are easily achieved by 10^5–10^6 neutrophils·ml^{-1} *in vitro*. Oxidative inactivation of proteinase inhibitors is one possible mechanism currently being discussed in the pathogenesis of lung emphysema. On the other hand, highly reactive OH^- radicals, mainly arising from metal-catalysed oxidation systems such as H_2O_2/Fe (II) or ascorbate/Fe(III) (Fenton-like reactions), cause damage to enzymes, fragmentation of connective tissue proteins, and lipid peroxidation. It is possible that, the myeloperoxidase/H_2O_2-system is also able to trigger lipid peroxidation [13]. These parameters reflect damage at the molecular level, giving a first insight into the mechanisms of oxidant-mediated lung injury. The real role of oxidants in the pathogenesis of lung diseases seems to be more complex, and is at

* GSF- Projekt Inhalation, Ingolstädter Landstrasse 1, D-8042 Neuherberg, FRG.

present not well understood in a number of inflammatory lung diseases.

In addition to the endogenously-generated reactive oxygen metabolites, the ubiquitous atmospheric pollutants, ozone and nitrogen dioxide, are potent oxidants. Exposure to ozone or nitrogen dioxide has been shown to produce alterations in a number of functional, biochemical, and morphological properties of the lung in humans and experimental animal models [14].

In order to protect the lung from increased endogenous or exogenous oxidant burden, several intracellular and extracellular antioxidant systems are available. They show different specificities in neutralizing toxic oxygen radicals. The delicate balance between oxidant toxicity and antioxidant systems (redox balance) is of major importance for maintaining normal lung function and structure. Disturbance of the redox balance through either increased oxidant production, reduced antioxidant capacity, or both, may favour pathophysiological events leading to acute or chronic damage and dysfunction. Superoxide dismutase removes the superoxide by dismutation to H_2O_2 and molecular oxygen, catalase detoxifies H_2O_2. Ceruloplasmin oxidizes Fe(II), which is known to be an efficient catalyst for OH^- formation in the Fenton reaction. Transferrin and lactoferrin bind extracellular iron (Fe(II)) in ELF and, thus, prevent formation of OH^-, which is a prerequisite for lipid peroxidation or modification of connective tissue proteins. Vitamin C is an antioxidant that can paradoxically also function as a pro-oxidant in certain conditions, *e.g.* in the presence of metal ions. It is present in most cells and in extracellular fluids, and seems to be selectively accumulated in the alveolar fluid. Vitamin E (α-tocopherol) is a lipid antioxidant principally located in cell membranes but also found in serum and normal human BAL fluid. Glutathione (GSH) is a thiol (SH)-containing tripeptide available in all cells and in ELF in high concentrations. Glutathione alone, and in concert with GSH peroxidase and GSH reductase, represents one of the most important antioxidant defence systems capable of scavenging OH^-, H_2O_2, products of the myeloperoxidase-catalysed reaction and of lipid peroxidation.

Glutathione concentrations in young asymptomatic cigarette smokers are 80% higher than in nonsmokers [15]. In contrast, ELF GSH concentrations in idiopathic pulmonary fibrosis reach only 25% of normal levels [16]. Depletion of GSH in ELF has also been found in cystic fibrosis and human immunodeficiency virus (HIV) infection. The significance of GSH as a scavenger of oxygen-derived free radicals has inaugurated therapeutic strategies to increase lung tissue levels of GSH: a) in GSH deficiency; and b) in lung diseases with an increased risk of oxidant injury, such as the adult respiratory distress syndrome or idiopathic pulmonary fibrosis. One approach proven to be effective in augmentation of GSH in ELF is through GSH aerosolization to the lower respiratory tract. This was first demonstrated in sheep [17], and more recently in idiopathic pulmonary fibrosis [18]. In the latter study, repeated administration of aerosolized reduced GSH significantly increased the levels of oxidized GSH in ELF in all of the patients studied.

Another antioxidant, which has received much attention in the last years is the thiol-containing drug N-acetylcysteine (NAC) [19, 20]. It is a powerful scavenger of OH^-, HOCl and N-chloramines [21], and several reports have shown that treatment with NAC (administered either intravenously or orally) is a promising approach to lower the risk of oxidant lung injury. Repeated administration of NAC increases plasma levels of cysteine and GSH [22, 23], and also augments ELF GSH in patients and normal subjects [20, 22–24].

In this issue of the journal, SALA *et al.* [25] report on the protective effect of NAC against oxidant lung injury induced by intrapulmonary immune complex deposition in rats. Serotonin uptake in the perfused lung served as a biochemical marker for pulmonary endothelial cell function. The authors found that loss of serotonin uptake as an indicator of cellular dysfunction was significantly reduced by 50% when 2 mmol NAC·kg^{-1} body weight was orally administered in a single dose, 30 min prior to starting immune complex deposition. This is an interesting animal study confirming the deleterious role of oxidants in immunological alveolitis, and showing the ability of NAC to support GSH synthesis in the lung. However, no results are shown on the metabolism of this drug, when administered at such a high dose. The kinetics of NAC decrease by deacetylation and the intermediate levels of cysteine in plasma and ELF are of special interest regarding possible adverse effects of this drug. For example, the function of the secretory leucocyte proteinase inhibitor, as one important component of the antiproteinase system in the lung, might be affected by the reduction of its disulphide bonds, caused by markedly elevated thiol-concentrations, resulting from antioxidant therapy with NAC. Thiols are generally considered to be antioxidants. However, they may also show a prooxidative effect, since reactive thiyl radicals may be formed from thiols *via* a mechanism which includes oxygen radicals. As recently demonstrated, thiyl radicals are able to induce peroxidation of polyunsaturated fatty acids, and may possibly cause lipid peroxidation in biological systems [26]. Another study has shown that cysteine at low concentrations (<1 mM) substantially promotes the inactivation of glucose-6-phosphate dehydrogenase by a metal-catalysed oxidation system consisting of Fe(II)/H_2O_2 *in vitro* (27). In conclusion, the administration of NAC in inflammatory lung diseases might enhance thiyl formation by endogenous oxygen radicals under certain conditions, representing an additional risk. Hence, further investigations seem to be necessary to evaluate this risk during antioxidant therapy.

References

1. Bellavitte P. – The superoxide-forming enzymatic system of phagocytes. *Free Rad Biol Med* 1988; 4: 225–261.
2. Halliwell B, Gutteridge JMC. – Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 1984; 219: 1–14.
3. Ramos CL, Pou S, Britigan BE, Cohen MS, Rosen GM. – Spin trapping evidence for myeloperoxidase-dependent

hydroxyl radical formation by human neutrophils and monocytes. *J Biol Chem* 1992; 267: 8307–8312.
4. Meier B, Cross AR, Hancock JT, Kaup FJ, Jones OTG. – Identification of a superoxide-generating NADPH oxidase system in human fibroblasts. *Biochem J* 1991; 275: 241–245.
5. Wallaert B, Aerts C, Gressier B, *et al.* – Ability of type II pneumocytes to release sufficient amounts of free radicals to oxidatively inactivate alpha$_1$-proteinase inhibitor in the presence of myeloperoxidase. *Eur Respir J* 1991; 4 (Suppl. 14): 520s.
6. Heffner JE, Repine JE. – Pulmonary strategies of antioxidant defense. *Am Rev Respir Dis* 1989; 140: 531–554.
7. Weiland JE, Davis WB, Holter JF, *et al.* – Lung neutrophils in the adult respiratory distress syndrome: clinical and pathophysiologic significance. *Am Rev Respir Dis* 1986; 133: 218–225.
8. Cantin AM, North SL, Fells GA, Hubbard RC, Crystal RG. – Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. *J Clin Invest* 1987; 79: 1665–1673.
9. Tonnel AB, Wallaert B. – Oxidants and bronchial inflammatory processes. *Eur Respir J* 1990; 3: 987–988.
10. Maier KL, Leuschel L, Costabel U. – Increased oxidized methionine residues in BAL fluid proteins in acute or chronic bronchitis. *Eur Respir J* 1992; 5: 651–658.
11. Roum JH, Buhl R, McElvaney NG, *et al.* – Cystic fibrosis is characterized by a marked reduction in glutathione levels in pulmonary epithelial lining fluid. *Am Rev Respir Dis* 1991; 141 (Suppl.): A87.
12. Oldham KT, Guice KS, Ward PA, Johnson KJ. – The role of oxygen radicals in immune complex injury. *Free Rad Biol Med* 1988; 4: 387–397.
13. Stelmaszynska T, Kukovetz E, Egger G, Schaur RJ. – Possible involvement of myeloperoxidase in lipid peroxidation. *Int J Biochem* 1992; 24: 121–128.
14. Evans MJ. – Oxidant gases. *Environ Health Perspect* 1984; 55: 85–95.
15. Cantin AM, North SL, Hubbard RC, Crystal RC. – Normal alveolar epithelial lining fluid contains high levels of glutathione. *J Appl Physiol* 1987; 63: 152–157.
16. Cantin AM, Hubbard RC, Crystal RG. – Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract in idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 1989; 139: 370–372.
17. Buhl R, Vogelmeier C, Critenden M, *et al.* – Augmentation of glutathione in the fluid lining the epithelium of the lower respiratory tract by directly administering glutathione aerosol. *Proc Natl Acad Sci USA* 1990; 87: 4063–4067.
18. Borok Z, Buhl R, Grimes GJ, *et al.* – Effect of glutathione aerosol on oxidant-antioxidant imbalance in idiopathic pulmonary fibrosis. *Lancet* 1991; 338: 215–216.
19. Patterson CE, Butler JA, Byrne FD, Rhodes ML. – Oxidant lung injury: intervention with sulfhydryl reagents. *Lung* 1985; 163: 23–32.
20. Moldeus P, Cotgreave IA, Berggren M. – Lung protection by a thiol containing antioxidant: N-acetylcysteine. *Respiration* 1986; 50 (Suppl. 1): 31–42.
21. Aruoma OI, Halliwell B, Hoey BM, Butler J. – The antioxidant action of N-acetylcysteine: its reaction with hydrogen peroxide, hydroxyl radical, superoxide, and hypochlorous acid. *Free Rad Biol Med* 1989; 6: 593–597.
22. Drost E, Lannan S, Bridgeman MME, *et al.* – Lack of effect of N-acetylcysteine on the release of oxygen radicals from neutrophils and alveolar macrophages. *Eur Respir J* 1991; 4: 723–729.
23. Bridgeman MME, Marsden M, MacNee W, Flenley DC, Ryle AP. – Cysteine and glutathione concentrations in plasma and bronchoalveolar lavage fluid after treatment with N-acetylcysteine. *Thorax* 1991; 46: 39–42.
24. Meyer A, Magnussen H. – The effect of oral N-acetylcysteine on glutathione levels in BAL of patients with idiopathic pulmonary fibrosis. *Am Rev Respir Dis* 1991; 143 (Suppl.): A736.
25. Sala R, Moriggi E, Corvasce G, Morelli D. – Protective effects of N-acetylcysteine against pulmonary endothelial cell damage induced by oxidant lung injury. *Eur Respir J* 1993; 6: 440–446.
26. Schöneich C, Dillinger U, von Bruchhausen F, Asmus KD. – Oxidation of polyunsaturated fatty acids and lipids through thiyl and sulfonyl radicals: reaction kinetics, and influence of oxygen and structure of thiyl radicals. *Arch Biochem Biophys* 1992; 292: 456–467.
27. Maier K, Hinze H, Leuschel L. – Prevention and promotion of oxidative mechanisms by sulphhydryl compounds *in vitro*: implication for the lungs. *Eur Respir J* 1992; 5 (Suppl. 15): 63s.