MECHANISMS OF NITRIC OXIDE INDUCED INJURY TO THE ALVEOLAR EPITHELIUM

Sha Zhu[1], Machelle Manuel[2], Imad Haddad[3] and Sadis Matalon[1]

[1]Department of Anesthesiology, University of Alabama at Birmingham, Birmingham, AL 35233, USA
[2]Department of Pharmacology, Texas Tech University Health Sciences Center, Amarillo, TX 79106, USA
[3]University of Minnesota, Division of Pulmonary & Critical Care, Minneapolis, MN 55455, USA

INTRODUCTION

The major function of the lung is gas exchange. The movement of both oxygen and carbon dioxide across the blood-gas barrier is by simple diffusion. This process is optimized by the large alveolar surface area, the close proximity of the alveolar and pulmonary capillary membranes, and the lack of any significant amount of fluid in the alveolar space. The relative dryness of the alveolar space is thought to be the result of: (1) the low permeability of the alveolar epithelium to both electrolytes and plasma proteins; (2) the presence of pulmonary surfactant, which lowers the surface tension of the blood-gas interface; and (3) the ability of alveolar epithelial cells to actively transport sodium ions from the alveolar to the basolateral spaces[1].

The alveolar epithelium is continuously exposed to both endogenously and exogenously derived sources of reactive oxygen and nitrogen species. These reactive species are formed as intermediates in mitochondrial electron-transport systems and microsomal metabolism of endogenous compounds and xenobiotics, including drugs and environmental pollutants and various cytoplasmic sources[2]. In addition, neutrophils and other inflammatory cells generate and release reactive oxygen species via an NADPH oxidase-dependent mechanism, which is mediated by membrane receptor activation of protein kinase C and phospholipase C. Prolonged and continuous exposure to reactive oxygen species damages the pulmonary surfactant system and alveolar epithelium resulting in increased amounts of protein in the alveolar space, pulmonary atelectasis, arterial hypoxemia and eventually death from respiratory failure. Herein we will review the biochemistry of the reactive oxygen and nitrogen species, the basic mechanisms by which they interact with target molecules in the alveolar epithelium and the short and long-term sequelae of these interactions.

Acute Respiratory Distress Syndrome: Cellular and Molecular Mechanisms and Clinical Management
Edited by Matalon and Sznajder, Plenum Press, New York, 1998

BIOCHEMISTRY OF REACTIVE OXYGEN SPECIES

Under normal oxygen tensions, approximately 98% of oxygen undergoes a four-electron catalytic reduction to form water by mitochondrial cytochrome c oxidase. The remaining 2% of oxygen, however, may undergo sequential incomplete reduction to form reactive oxygen species such as superoxide ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2). Both $O_2^{\cdot-}$ and H_2O_2 are relatively long-lived compounds in biologic systems. H_2O_2 can directly cross cell membranes by simple diffusion, while $O_2^{\cdot-}$ crosses cell membrane via anion channels. However, the limited reactivity of $O_2^{\cdot-}$ and H_2O_2 with many biological molecules and their very low intracellular concentrations (10 pM and 1-100 nM, respectively) have raised questions about their toxicity *per se*.

Several factors may exacerbate production of reactive oxygen species in acute and chronic lung diseases. First, increased oxygen concentration is commonly required to alleviate arterial and tissue hypoxemia in patients with pulmonary and cardial diseases. Exposure of lung cells, subcellular organelles, and tissue to hyperoxia (100% O_2) has been shown to increase mitochondrial hydrogen peroxide production 10-15 fold [3]. Second, in response to pro-inflammatory cytokines, activated neutrophils and macrophages migrate to the lungs and release reactive oxygen species by the membrane-bound enzyme-complex NADPH oxidase [4]. Third, under conditions of ischemia, decreased perfusion, low oxygen tension, or trauma, xanthine dehydrogenase, the innocuous form of the enzyme, is converted to xanthine oxidase, which utilizes xanthine and molecular oxygen to produce partially reduced oxygen species (PROS). The results of several studies suggest that xanthine oxidase may be released from the intestine or liver into the circulation and bind to pulmonary endothelium where it can serve as a locus for the intense production of reactive oxygen species[5].

A more potent reactive metabolite of $O_2^{\cdot-}$ is the hydroxyl radical ($^{\cdot}OH$). Superoxide anions directly reduce H_2O_2 to give O_2, hydroxide ion (OH^-) and $^{\cdot}OH$. In the presence of trace metals (usually Fe^{3+}, sometimes Cu^{2+}), $^{\cdot}OH$ can be generated via the following pathways:

$$O_2^{\cdot-} + Fe^{3+} \rightarrow Fe^{2+} + O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\cdot}OH$$

$$O_2^{\cdot-} + H_2O_2 \rightarrow O_2 + OH^- + {}^{\cdot}OH$$

Hydroxyl radicals are much more potent oxidants than either $O_2^{\cdot-}$ or H_2O_2 and are capable of producing extensive cellular damage. Their reactivity is so high and nonspecific that the site of target reaction is confined to within a few molecular radii of the site of its generation. There are no direct enzymatic scavenging systems present *in vivo* for this radical. *In vitro*, the presence of OH is revealed by the inhibition of its formation by the scavenging action of mannitol, ethanol or dimethyl sulfoxide (DMSO).

MOLECULAR BIOLOGY OF $^{\cdot}NO$

Nitric Oxide Synthase

Generation of $^{\cdot}OH$ by the Fenton reaction requires the interaction of three different species (O_2, H_2O_2 and Fe^{3+}). In the epithelial lining fluid the concentrations of reactive oxygen species are kept low due to the presence of the antioxidant enzymes superoxide

dismutase and catalase, along with a number of non-enzymatic antioxidants, including vitamin E, reduced glutathione and ascorbate [6]. Furthermore, most iron is chelated in a non-catalytic form by transferrin and ceruloplasmin. Although the formation of ·OH via the Fenton reaction *in vivo* may still occur, especially in situations where the intracellular load of free iron has been increased [7,8], a second pathway for the generation of potential oxidants with the reactivity of ·OH without the need for metal catalysis, involving nitric oxide (·NO) has recently been described [9].

·NO is synthesized from the five electron oxidation of either of the two equivalent guanidine nitrogens of L-arginine. The reaction is catalyzed by one of three isozymes of nitric oxide synthase (NOS), using reduced NADPH as the source of electrons and cofactors, including tetrahydrobiopterin (H_4B) and flavin nucleotides (FMN; FAD). Molecular oxygen is a co-substrate, N^G-hydroxy-L-arginine is formed as a short-lived intermediate and L-citrulline is a byproduct. All isoforms of NOS bind calmodulin and contain heme. Electrons are supplied by NADPH, transferred along the flavins and calmodulin, and presented to the catalytic heme[10].

Our current understanding of how ·NO performs an extraordinarily diverse array of physiologic and pathophysiologic functions remains quite rudimentary. Nonetheless, it appears that regulation of ·NO biosynthesis explains, in part, its diverse functions, and thus significant advances have been made studying unique characteristics of NOS isoforms. Collectively, NOS are homodimeric cytochrome P450-like hemoproteins that have oxygenase and reductase domains in their amino (NH_2) and carboxyl (COOH) termini respectively. The active dimeric form is hypothesized to be dependent upon H_4B. The domains are separated by a Ca^{2+}/calmodulin binding region. The reductase domain is homologous to NADPH-cytochrome P450 including binding sites for FMN, FAD and NADPH. The constitutive forms have similar phosphorylation sites and one of them (NOS-III; see below) has a unique NH_2 terminal myristolyation site.

Differences between the isozymes, however, underscore the function of ·NO in various biological systems. The nomenclature evolved from descriptions of cellular source of the enzyme (e.g., neuronal, macrophage or endothelial) or its expression (constitutive vs inducible) and by many conventions is now relegated to the chronology in which the enzyme was purified and cloned. NOS I and III are constitutively expressed and their activity is regulated by intracellular calcium; prototypic sources are neuronal and endothelial cell, respectively[10]. NOS II is induced by cytokines, its activity is largely independent of calcium and it is regulated at a transcriptional level. The prototypic source is the macrophage [11,12]. Although the human genes are present on discrete chromosomes, considerable homology exists between the three isoforms suggesting common ancestral origin with subsequent gene duplication and transposition. NOS-I and III derived ·NO is produced in small quantities for brief periods of time and underscore intra-and intercellular signaling events such as neurotransmission or vascular homeostasis. In contrast, NOS-II produces large amounts of ·NO for prolonged periods of time (assuming availability of substrate and cofactors) and contributes to more diffuse physiological roles associated with inflammation or infection.

PHYSIOLOGICAL EFFECTS OF ·NO

Biological Targets

The biologic actions of ·NO are dictated by the reactions it undergoes with different target molecules in cells, membranes, and extracellular milieu. For each target, depending on the amount and duration of induction, ·NO can exert beneficial or detrimental effects. Known targets for ·NO include:

Guanylate cyclase. ·NO binds the heme group of soluble guanylate cyclase leading to an increase in cyclic guanosine-3',5'-monophosphate (cGMP) levels. Many effects of cGMP are mediated via a group of enzymes, cGMP-associated protein kinases (PKG's). These PKGs act to reduce intracellular calcium causing smooth muscle relaxation. ·NO-mediated increased cGMP levels also prevents platelets aggregation, and decreases adhesion of neutrophils. However, excessive ·NO-mediated cGMP production has been implicated in sepsis-induced refractory hypotension and shock [13].

Hemoglobin. The major route for the destruction of ·NO *in vivo* is the fast and irreversible reaction with *oxy*-hemoglobin (Hb-$Fe^{2+}O_2$) or *oxy*-myoglobin to produce nitrate and methemoglobin, according to the following reactions:

$$Hb\text{-}Fe^{2+}O_2 + {\cdot}NO \rightarrow Hb\text{-}Fe^{2+}ONOO \rightarrow Hb\text{-}Fe^{3+} + NO_3^-$$

Because of its vasorelaxant properties and rapid inactivation in the blood by its reaction with hemoglobin, ·NO inhalation has been advocated as a means of selectively reducing pulmonary hypertension and improving systemic oxygenation in a variety of clinical situations including bronchopulmonary dysplasia, and the acute respiratory distress syndrome (ARDS) [14]. However, patients with diminished methemoglobin reductase activity, such as neonates, are unable to efficiently convert methemoglobin to ferrous hemoglobin, and could therefore be at greater risk for developing methemoglobinemia and decreased oxygen transport. Recent reports indicate that the reversible S-nitrosylation of a cysteine residue located in the β-chain (Cysβ93) plays an important role in optimizing oxygen delivery to the tissues [15].

Iron/sulfur (4Fe/4S) centers of enzymes. Production of ·NO by activated macrophages defends the host against infectious agents, including bacteria, parasites, viruses, and destroys tumor cells. The proposed mechanisms responsible for these effects involve the reaction of ·NO with the nonheme iron of iron-sulfur complexes, resulting in the inactivation of iron-sulfur containing enzymes including mitochondrial aconitase, cytochrome c oxidase [16], and the DNA synthesis rate limiting enzyme, ribonucleotide reductase. Inhibition of these critical enzymes leads to suppression of mitochondrial respiration, energy metabolism and cell replication. However, the ·NO effects are nonspecific and its overproduction may be cytotoxic not only for microbes, but also for the cells and tissues that produce it [17].

Other free radicals. NO has an unpaired electron, and thus can readily react with other free radicals, as specified below:

a. Reaction with oxygen. If a tank of ·NO is allowed to leak into air, a cloud of lethal and highly reactive orange-brown nitrogen dioxide (·NO_2) is formed, according to the following reactions:

$$2\ {\cdot}NO + O_2 \rightarrow 2\ {\cdot}NO_2$$

At low ·NO concentrations (<1-2 μM), observed *in vivo* at most pathologic conditions, the low probability of any two ·NO molecules encountering each other makes the formation of ·NO_2 extremely slow. Instead, ·NO may react with a single molecule of oxygen is a second order reaction to form a nitrosyldioxyl radical (ONOO·).

b. Reaction with $O_2^{\cdot-}$. ·NO reacts with $O_2^{\cdot-}$ at a near diffusion-limited rate constant of about 7 x 10^9 $M^{-1}s^{-1}$ to form $ONOO^-$. This reaction was mistakenly thought to be

protective since it decreased the amount of $O_2^{\cdot-}$ detected. ONOO- has a pK_a of 6.8 at 37°C and thus may remain stable for months in alkaline solutions. The protonated form of peroxynitrous acid (ONOOH) forms $^{\cdot}NO_2$ and an intermediate with a reactivity equivalent to the hydroxyl radical, derived from the *trans* isomerization of ONOOH:

$$O_2^{\cdot-} + {}^{\cdot}NO \rightarrow ONOO^- + H^+ \rightarrow ONOOH \rightarrow \begin{cases} \text{"}{}^{\cdot}OH \cdots {}^{\cdot}NO_2\text{"} \\ NO_3^- \end{cases}$$

Under physiological conditions, a minimum of 25% of $ONOO^-$ decomposes to form the "$^{\cdot}OH \cdots {}^{\cdot}NO_2$", with the remainder recombining to form nitrate. Thus peroxynitrite may serve as a source for $^{\cdot}OH$ type species without the requirement of metal catalysis[9].

While being highly reactive, its modest rate of decomposition under physiological conditions allows $ONOO^-$ to diffuse for up to several cell diameters to critical cellular targets before, becoming protonated and decomposing. $ONOO^-$ initiates iron-independent lipid peroxidation and oxidizes thiols, damages the mitochondria electron transport chain [18], and causes lipid peroxidation of human low density lipoproteins. In addition, metal ions, such as Fe^{3+}EDTA and copper in the active site of superoxide dismutase (SOD), catalyze the heterolytic cleavage of $ONOO^-$ to form a nitronium ion-like species (NO_2^+) which nitrates phenolics including tyrosine in proteins [19].

Under normal conditions, intracellular $O_2^{\cdot-}$ concentrations are kept at remarkably low levels (10 pM) because eukaryotic cells contain large amounts of SOD (4-10 μM). Under these conditions, $ONOO^-$ formation is minimal. However, inflammatory cells produce large number of both $^{\cdot}NO$ and $O_2^{\cdot-}$ when stimulated by cytokines, interferon γ, LPS, and other inflammatory agents . When the concentration of $^{\cdot}NO$ increases to the micromolar range, it can effectively compete with SOD for $O_2^{\cdot-}$ to form $ONOO^-$ (rate constant of the reaction of $O_2^{\cdot-}$ with $^{\cdot}NO$ is three times faster than that with SOD). Using luminol-dependent chemiluminescence, $ONOO^-$ production has been demonstrated by human neutrophils [20], rat alveolar macrophages [12], and bovine aortic endothelial cells [21]. Furthermore, airway epithelial cells constitutively express iNOS[22].

One may speculate that low molecular weight antioxidants (such as reduced glutathione and ascorbate) present in the epithelial lining fluid may scavenge peroxynitrite thus preventing its interaction with biological targets. However, because of its high reactivity, $ONOO^-$ will attack biological targets even in the presence of antioxidant substances [23]. Furthermore, physiological concentrations of carbon dioxide and bicarbonate enhance the reactivity of $ONOO^-$ via the formation of the nitrosoperoxycarbonate anion ($O{=}N{=}OOCO_2^-$) and increase its nitration efficiency[24,25]. Equally important, bicarbonate reversed the inhibition of $ONOO^-$-induced nitration by ascorbate and urate[25]. The detection of nitrotyrosine in the lungs of patients with adult respiratory distress syndrome (ARDS) [26] and lungs of rats exposed to endotoxin [27] or hyperoxia [26] indicates that nitration reactions occur *in vivo*.

Recently, a new pathway for the formation of nitrating species has been proposed. Eiserich et al. [28]showed that the reaction of nitrite (NO_2^-), the autoxidation product of $^{\cdot}NO$, with hypochlorous acid (HOCl) forms reactive intermediate species with spectral characteristics similar to those of nitryl chloride ($Cl\text{-}NO_2$), that are also capable of nitrating phenolic substrates such as tyrosine and 4- hydroxyphenylacetic acid, with maximum yields obtained at physiological pH. This reaction may have considerable physiological significance since considerable amounts of HOCl are being produced by activated neutrophils via the action of myeloperoxidase on hydrogen peroxide. Recent observations indicate a six-fold elevation of 3-chlorotyrosine in atherosclerotic tissue obtained during vascular surgery as compared to normal aortic intima. The detection of 3-chlorotyrosine in human atherosclerotic lesions indicates that halogenation reactions catalyzed by the myeloperoxidase system of phagocytes constitute one pathway for protein oxidation in vivo[29].

c. Thiols. It has been suggested that various forms of NO (such as N_2O_3, NO^+, or $ONOO^-$) may react with thiols to yield S-nitrosothiols (RS-NO) and that NO circulates in plasma mainly as an S-nitroso adduct of serum albumin. However, the exact biochemical pathways leading to S-nitrosothiol formation are not clear. NO will not react directly with a thiol (RSH) as the reaction is unbalanced and thermodynamically unfavorable. NO will react with a one electron acceptor, such as iron, nitrogen dioxide or as recently suggested, molecular oxygen[30], to form a nitrosonium ion (NO^+), which can then interact with thiols to form nitrosothiols. Micromolar concentrations of S-nitrosoglutathione have been detected in the airway fluid of normal subjects and significantly higher levels were observed in the lungs of patients with pneumonia or during inhalation of 80 ppm NO [31]. It has been suggested that formation of RS-NO adducts stabilizes NO, decreasing its cytotoxic potential, while maintaining its bioactive properties. NO can also be transported on cysteine residues of hemoglobin which may facilitate efficient delivery of oxygen to tissues [15].

$ONOO^-$ Formation in ARDS

ARDS, triggered by a number of pathologic conditions, is a clinical syndrome that features severe lung inflammation with abnormal permeability of the alveolar epithelium. The edema is a result of injury to both endothelial and epithelial cells caused by reactive species and proteolytic enzymes released by activated neutrophils and alveolar macrophages. Despite the identification of many mediators which lead to neutrophil tissue infiltration and activation, overall mortality from ARDS remains at 50-70%. The lack of specific treatment for ARDS is due to the complex interplay between the different humoral mediators released by the initiating condition.

The following observations establish the potential involvement of ANO, $ONOO^-$ and various reactive nitrogen species in the generation and propagation of pulmonary epithelial injury in a variety of ARDS-type pathological conditions: (1) Induction of immune complex alveolitis in rat lungs results in significant elevation of ANO decomposition products and albumin levels in the bronchoalveolar lavage (BAL), indicating the presence of increased alveolar permeability to solute. Alveolar instillation of L-NMMA mitigates ANO production and alveolar epithelial injury [32]. (2) Paraquat induced injury to the lung results in stimulation of ANO synthesis. All signs of injury, including increased airway resistance and alveolar permeability to solute, are mitigated by administration of selective and competitive inhibitors of nitric oxide synthase [33]. (3) Ischemia-reperfusion injury to isolated rat lungs is associated with an increase in protein nitrotyrosine in lung homogenates using amino acid analysis, increased nitrate and nitrite levels in perfusate fluid, and formation of tissue oxidized protein and lipid products. Administration of L-NAME (NOS inhibitor) 30 min prior to induction of ischemia abolishes the increases in both nitrotyrosine and nitrate and nitrite, and significantly reduces the formation of lung thiobarbituric acid reactive substances (TBARS) and protein carbonyl levels [34]. (4) Infecting hamster tracheal rings with *Bordetella pertussis in vitro* produces epithelial cytopathology. Destruction of ciliated cells and inhibition of DNA synthesis are associated with induction of NO synthesis by the tracheal epithelial cells. The cytopathology is dramatically attenuated by the NOS inhibitors L-NMMA and aminoguanidine. These results indicate that pertussis toxins elicits NO production in the same cells that suffer the subsequent deleterious effects [17]. (5) Pneumonia due to influenza virus involves increased production of both NO and O_2^- [35]. Increased expression and activity of NOS-II is observed in lungs infected with the influenza virus. L-NMMA, administered intraperitoneally daily to mice from day 3 after virus inoculation improves survival.

One way to demonstrate $ONOO^-$ formation *in vivo* is to detect the presence of stable by-products of its reaction with various biological compounds. 3-Nitro-tyrosine, the product of the addition of a nitro group ($-NO_2$) to the *ortho* position of the hydroxyl group of tyrosine, is such a stable compound. Using a polyclonal antibody, which recognizes antigenic sites related to nitrotyrosine [26], we demonstrated increased immunostaining in the lung of pediatric patients who died with ARDS and in the lungs of rats exposed to sublethal hyperoxia (100% O_2 for 60 h). Immunostaining was specific since it was blocked by the addition of an excess amount of antigen, and was absent when the nitrotyrosine antibody was replaced with non-specific IgG. Nitrotyrosine formation was detected only in rat lung sections incubated *in vitro* with $ONOO^-$, but not $^{\cdot}NO$ or reactive oxygen species. The most likely candidate capable of nitrating tyrosine residues is $ONOO^-$. Thus, this data suggest that $ONOO^-$ is formed in the lungs of patients and animals with acute lung injury.

However, $ONOO^-$ may not be the only species capable of tyrosine nitration. $^{\cdot}NO_2$ can also nitrate tyrosine, although it is much less efficient than $ONOO^-$ because two molecules of $^{\cdot}NO_2$ are required to nitrate one tyrosine. Another possible nitration pathway, as mentioned previously, is the reaction of $^{\cdot}NO$-derived nitrite, under acidic conditions with oxidants such as H_2O_2 and hypochlorous acid to form the nitrating agents [36].

Physiological Consequences of Protein Nitration

Several reports indicate that protein nitration may lead to selective loss of protein function. Nitration of tyrosine residues of human IgG, abrogated their C_{1q}-binding activity [37]. The inactivation of *E. coli* dUTPase and the occurrence of a tyrosine residue in a strictly conserved sequence motif, suggest the critical importance of this residue for the function of the enzyme [38]. Nitration of tyrosine residues of α_1-proteinase inhibitor resulted in selective loss of elastase inhibitory activity, but not chymotrypsin or trypsin-inhibitory activity [39]. Exposure of surfactant protein A (SP-A) to peroxynitrite or specific nitrating agents led to nitration of a single tyrosine residue in its carbohydrate recognition domain and diminished the ability of SP-A to aggregate lipids and bind to mannose [40-42]. SP-A, isolated from the lungs of lambs breathing $^{\cdot}NO$ and O_2 also had decreased ability to aggregate lipids [43]. In contrast, exposure of SP-A to strong oxidizing agents, which by themselves do not nitrate tyrosines, did not alter SP-A function. Tyrosine nitration has also been shown to inhibit protein phosphorylation by tyrosine kinases, and thus interfere with intracellular signal transduction [44,45]

Injury to pulmonary surfactant and alveolar epithelium *in vivo*

Because of its vasorelaxant properties and its rapid inactivation in the blood by its reaction with hemoglobin, $^{\cdot}NO$ inhalation has been advocated as a means of selectively reducing pulmonary hypertension and improving systemic oxygenation in a variety of clinical situations including bronchopulmonary dysplasia and ARDS. However there is concern that inhalation of $^{\cdot}NO$ in the presence of acute inflammation may lead to the formation of reactive oxygen-nitrogen species that may damage the alveolar epithelium and pulmonary surfactant system.

A number of recent reports seem to indicate that $^{\cdot}NO$ inhalation may indeed damage the lungs. Exposure of newborn piglets to 100 ppm $^{\cdot}NO$ and 95% O_2 for 48 h resulted in significant injury to pulmonary surfactant, manifested by inhibition of surface activity and worsened pulmonary inflammation [46]. Pulmonary surfactant samples isolated from newborn lambs exposed to $^{\cdot}NO$ gas (200 ppm) for 6 h exhibited abnormal surface properties. SP-A,

isolated from the lungs of lambs that breathed 200 ppm NO, exhibited a small, but significant decrease in the ability to aggregate lipids *in vitro*[43]. Hallman et al. [47] reported that exposure of rats to 100 ppm NO in 95% O_2 for 24 h developed surfactant dysfunction caused in part by alterations of proteins in the epithelial lining fluid.

It may be argued that the concentrations of inhaled NO in these studies were outside the range used clinically. However, due to the short exposure period utilized in these experiments, the value of the product of *concentration x time* of inhaled NO is comparable to the corresponding value in a patient who breathes 20 ppm NO for 3 d. Exposure of rats to 0.5 ppm NO for 9 weeks resulted in significantly higher injury to lung interstitial cells and matrix than an equivalent exposure to NO_2, implicating NO as an agent more toxic than NO_2 [48]. None of the animals showed overt evidence of pulmonary injury such as arterial hypoxemia, increased albumin content in the BAL or respiratory failure. Accordingly, prolonged inhalation of NO in ARDS may lead to subacute lung injury that may compound the existing pathology.

SUMMARY AND CONCLUSIONS

Oxidant stress affects virtually all aspects of biologic existence by reaction with, and modification of, structural, metabolic, and genetic material. Protective mechanisms have evolved to defend cell components, but disease states, and other environmental stresses can overwhelm defense mechanisms and cause cytotoxicity. The discovery of the L-arginine-NO pathway has modified our understanding of the nature of the injurious species and the role NO plays in oxidant stress in the lung. There is no argument that inhalation of NO has been proven efficacious in improving oxygenation in infants with idiopathic pulmonary hypertension and in a variety of other disorders. Furthermore, in some cases, NO may actually reduce oxidant lung injury by preventing the propagation of lipid peroxidation. However, the reaction of NO with superoxide has been shown to produce peroxynitrite, a very reactive species capable of damaging the pulmonary surfactant and the alveolar epithelium. Thus the biological action of NO may depend on what type of molecule it is interacting with. Recent studies indicating that inhaled NO injury to the alveolar epithelium may be reduced by intratracheal instillation of antioxidant enzymes [49]offer promise in developing novel approaches to minimize its toxicity and thus enhance its therapeutic potential.

Acknowledgments

This work was supported by grants from the National Institutes of Health (HL31197 and HL51173), and a grant from the Office of Naval Research (N00014-97-1-0309).

References

1. Matalon, S., Benos, D.J. & Jackson, R.M. *Am.J.Physiol.* **271**, L1-22 (1996).
2. Freeman, B.A. & Crapo, J.D. *Lab.Invest.* **47**, 412-426 (1982).
3. Turrens, J.F., Freeman, B.A. & Crapo, J.D. *Arch.Biochem.Biophys.* **217**, 411-421 (1982).
4. Babior, B.M. *Environ.Health Perspect.* **102 Suppl 10**, 53-56 (1994).
5. Weinbroum, A., Nielsen, V.G., Tan, S., et al. *Am.J.Physiol.* **268**, G988-96 (1995).
6. Cantin, A.M., North, S.L., Hubbard, R.C. & Crystal, R.G. *J.Appl.Physiol.* **63**, 152-157 (1987).

7. Chao, C.C., Park, S.H. & Aust, A.E. *Arch.Biochem.Biophys.* **326**, 152-157 (1996).
8. Hardy, J.A. & Aust, A.E. *Carcinogenesis* **16**, 319-325 (1995).
9. Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A. & Freeman, B.A. *Proc.Natl.Acad.Sci.USA* **87**, 1620-1624 (1990).
10. Ignarro, L.J. *Annu.Rev.Pharmacol.Toxicol.* **30**, 535-560 (1990).
11. Kwon, N.S., Stuehr, D.J. & Nathan, C.F. *J.Exp.Med.* **174**, 761-767 (1991).
12. Ischiropoulos, H., Zhu, L. & Beckman, J.S. *Arch.Biochem.Biophys.* **298**, 446-451 (1992).
13. Wei, X.Q., Charles, I.G., Smith, A., et al. *Nature* **375**, 408-411 (1995).
14. Rossaint, R., Falke, K.J., Lopez, F., Slama, K., Pison, U. & Zapol, W.M. *N.Engl.J.Med.* **328**, 399-405 (1993).
15. Jia, L., Bonaventura, J. & Stamler, J.S. *Nature* **380**, 221-226 (1996).
16. Cleeter, M.W., Cooper, J.M., Darley-Usmar, V.M., Moncada, S. & Schapira, A.H. *FEBS Lett.* **345**, 50-54 (1994).
17. Heiss, L.N., Lancaster, J.R., Jr., Corbett, J.A. & Goldman, W.E. *Proc.Natl.Acad.Sci.USA* **91**, 267-270 (1994).
18. Radi, R., Rodriguez, M., Castro, L. & Telleri, R. *Arch.Biochem.Biophys.* **308**, 89-95 (1994).
19. Beckman, J.S., Ischiropoulos, H., Zhu, L., et al. *Arch.Biochem.Biophys.* **298**, 438-445 (1992).
20. Carreras, M.C., Pargament, G.A., Catz, S.D., Poderoso, J.J. & Boveris, A. *FEBS Lett.* **341**, 65-68 (1994).
21. Kooy, N.W. & Royall, J.A. *Arch.Biochem.Biophys.* **310**, 352-359 (1994).
22. Kobzik, L., Bredt, D.S., Lowenstein, C.J., et al. *Am.J.Respir.Cell Mol.Biol.* **9**, 371-377 (1993).
23. van der Vliet, A., Smith, D., O'Neill, C.A., et al. *Biochem.J.* **303**, 295-301 (1994).
24. Denicola, A., Freeman, B.A., Trujillo, M. & Radi, R. *Arch.Biochem.Biophys.* **333**, 49-58 (1996).
25. Gow, A., Duran, D., Thom, S.R. & Ischiropoulos, H. *Arch.Biochem.Biophys.* **333**, 42-48 (1996).
26. Haddad, I.Y., Pataki, G., Hu, P., Galliani, C., Beckman, J.S. & Matalon, S. *J.Clin.Invest.* **94**, 2407-2413 (1994).
27. Wizemann, T.M., Gardner, C.R., Laskin, J.D., et al. *J.Leukoc.Biol.* **56**, 759-768 (1994).
28. Eiserich, J.P., Cross, C.E., Jones, A.D., Halliwell, B. & van der Vliet, A. *J.Biol.Chem.* **271**, 19199-19208 (1996).
29. Hazen, S.L. & Heinecke, J.W. *J.Clin.Invest.* **99**, 2075-2081 (1997).
30. Gow, A.J., Buerk, D.G. & Ischiropoulos, H. *J.Biol.Chem.* **272**, 2841-2845 (1997).
31. Gaston, B., Reilly, J., Drazen, J.M., et al. *Proc.Natl.Acad.Sci.USA* **90**, 10957-10961 (1993).
32. Mulligan, M.S., Hevel, J.M., Marletta, M.A. & Ward, P.A. *Proc.Natl.Acad.Sci.USA* **88** , 6338-6342 (1991).
33. Berisha, H.I., Pakbaz, H., Absood, A. & Said, S.I. *Proc.Natl.Acad.Sci.USA* **91**, 7445-7449 (1994).
34. Ischiropoulos, H., al-Mehdi, A.B. & Fisher, A.B. *Am.J.Physiol.* **269**, L158-64 (1995).
35. Akaike, T., Noguchi, Y., Ijiri, S., et al. *Proc.Natl.Acad.Sci.USA* **93**, 2448-2453 (1996).
36. van der Vliet, A., Eiserich, J.P., O'Neill, C.A., Halliwell, B. & Cross, C.E. *Arch.Biochem.Biophys.* **319**, 341-349 (1995).

37. McCall, M.N. & Easterbrook-Smith, S.B. *Biochem.J.* **257**, 845-851 (1989).
38. Vertessy, B.G., Zalud, P., Nyman, O.P. & Zeppezauer, M. *Biochim.Biophys.Acta* **1205**, 146-150 (1994).
39. Feste, A. & Gan, J.C. *J.Biol.Chem.* **256**, 6374-6380 (1981).
40. Zhu, S., Haddad, I.Y. & Matalon, S. *Arch.Biochem.Biophys.* **333**, 282-290 (1996).
41. Haddad, I.Y., Crow, J.P., Hu, P., Ye, Y., Beckman, J. & Matalon, S. *Am.J.Physiol.* **267**, L242-9 (1994).
42. Haddad, I.Y., Zhu, S., Ischiropoulos, H. & Matalon, S. *Am.J.Physiol.* **270**, L281-8 (1996).
43. Matalon, S., DeMarco, V., Haddad, I.Y., et al. *Am.J.Physiol.* **270**, L273-80 (1996).
44. Kong, S.K., Yim, M.B., Stadtman, E.R. & Chock, P.B. *Proc.Natl.Acad.Sci.U.S.A.* **93**, 3377-3382 (1996).
45. Gow, A.J., Duran, D., Malcolm, S. & Ischiropoulos, H. *FEBS Lett.* **385**, 63-66 (1996).
46. Robbins, C.G., Davis, J.M., Merritt, T.A., et al. *Am.J.Physiol.* **269**, L545-50 (1995).
47. Hallman, M., Waffarn, F., Bry, K., et al. *J.Appl.Physiol.* **80**, 2026-2034 (1996).
48. Mercer, R.R., Costa, D.L. & Crapo, J.D. *Lab.Invest.* **73**, 20-28 (1995).
49. Robbins, C.G., Horowitz, S., Merritt, T.A., et al. *Am.J.Physiol.* **272**, L903-L907(1997).