Redox Report

Communications in Free Radical Research

ISSN: 1351-0002 (Print) 1743-2928 (Online) Journal homepage: https://www.tandfonline.com/loi/yrer20

Role of nitric oxide in pathological responses of the lung to exposure to environmental/ occupational agents

Patti C. Zeidler & Vincent Castranova

To cite this article: Patti C. Zeidler & Vincent Castranova (2004) Role of nitric oxide in pathological responses of the lung to exposure to environmental/occupational agents, Redox Report, 9:1, 7-18, DOI: 10.1179/135100004225003879

To link to this article: https://doi.org/10.1179/135100004225003879

Review

Role of nitric oxide in pathological responses of the lung to exposure to environmental/occupational agents

Patti C. Zeidler, Vincent Castranova

Department of Physiology and Pharmacology, West Virginia University and National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

Conflicting evidence exists as to whether nitric oxide expresses damaging/inflammatory or antioxidant/anti-inflammatory properties. Data presented in this review indicate that *in vitro* or *in vivo* exposure to selected environmental or occupational agents, such as asbestos, silica, ozone or lipopolysaccharide, can result in up-regulation of inducible nitric oxide synthase by alveolar macrophages and pulmonary epithelial cells. In the case of silica exposure, evidence consistently supports a damaging/inflammatory role of nitric oxide and/or peroxynitrite in the pathogenesis of lung disease. Although conflicting data have been reported, the majority of published studies suggest that nitric oxide plays a damaging role in pulmonary injury resulting from exposure to ozone or asbestos. In contrast, most information supports an anti-inflammatory role of nitric oxide following exposure to lipopolysaccharide. Further investigation is required to elucidate fully the mechanisms involved in determining the role of nitric oxide in the initiation and progression of various pulmonary diseases.

Keywords: Nitric oxide, lung pathology, asbestos, silica, ozone, lipopolysaccharide, alveolar macrophages, pulmonary epithelial cells

INTRODUCTION

Nitric oxide (NO) can be generated by a cytosolic enzyme, nitric oxide synthase (NOS), according to the following reaction:

$$\text{L-Arginine} + \text{NADPH} \xrightarrow{\text{NOS}} \text{L-Citrulline} + \text{NO} \qquad \text{Eq. 1}$$

Neuronal NOS (nNOS or NOS1), present in nervous tissue, and endothelial NOS (eNOS or NOS3), present in vascular endothelium, are isoforms of constitutive NOS and are calcium/calmodulin dependent. Constitutive forms of NOS produce NO at low basal rates and are involved in vasorelaxation and neurotransmission. A third isoform is induced by a variety of stimuli, such as lipopolysaccharide, interferon-γ (IFN-γ), chemotactic peptide, platelet activating factor, leukotriene B_4, and selected cytokines. Inducible NOS (iNOS or NOS2) is tightly bound to calmodulin and is active at basal, cytosolic calcium levels. Therefore, it was originally described as calcium/calmodulin independent. iNOS can generate relatively high levels of NO, which have been associated with a wide range of biological responses.[1–3]

Constitutive and inducible isoforms of NOS have been identified in various lung cell types (Table 1). Under basal conditions, eNOS has been found in alveolar macrophages, alveolar type II cells, and pulmonary endothelial cells.[4–6] Type II cell eNOS appears to be more active than that

Received 23 July 2003
Accepted 25 September 2003

Correspondence to: Vincent Castranova PhD, Chief, Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, M/S L-2015, 1095 Willowdale Road, Morgantown, WV 26505, USA
Tel: +1 304 285 6056; Fax: +1 304 285 5938; E-mail: vic1@cdc.gov

Abbreviations: IFN-γ, interferon gamma; LPS, lipopolysaccharide; IL-1, interleukin-1; TNF-α, tumor necrosis factor alpha; eNOS and NOS3, endothelial NOS; nNOS and NOS1, neuronal NOS; iNOS and NOS2, inducible NOS; BALF, bronchoalveolar lavage fluid; MPO, myeloperoxidase; MIP-2, macrophage inflammatory protein-2; IL-6, interleukin-6; BALC, bronchoalveolar lavage cells; NOx, nitrate and nitrite; NO, nitric oxide, $O_2^{\bullet-}$, superoxide anion; AM, alveolar macrophages; MMP-9, matrix metalloproteinase-9; NF-κB, nuclear factor-kappa B; PGE_2, prostaglandin E_2

DOI 10.1179/135100004225003879

Table 1. Types of NOS found in lung cells

Lung cell	NOS isoform	Condition	Presence	Reference
Alveolar macrophages	eNOS	Basal	Low	4
	nNOS	Basal	No	4
	iNOS	Basal	No	4
	iNOS	IFN-γ	High	9
Interstitial macrophages	iNOS	IFN-γ/LPS	High	10
Type II cells	eNOS	Basal	Moderate	5
	nNOS	Basal	No	5
	iNOS	Basal	No	8
	iNOS	IFN-γ or IL-1	Moderate	8
Fibroblasts	iNOS	IFN-γ and/or IL-1β	High	11
Pulmonary arterial smooth muscle cells	iNOS	Cytomix (IL-1β, TNF-α, IFN-γ, LPS)	High	12
Pulmonary endothelial cells	eNOS	Basal	Low	6
Airway neurons	nNOS	Basal	Low	7
Neutrophils	iNOS	Chemotactic factor, platelet activating factor, or leukotriene B_4	High	3

found in alveolar macrophages. In contrast, nNOS has been found in airway neurons but not in alveolar macrophages or alveolar type II cells.[4,5,7] iNOS has not been identified in unstimulated alveolar macrophages or type II cells.[4,8] However, various stimulants have been reported to induce the expression of iNOS in alveolar macrophages, type II cells, interstitial macrophages, fibroblasts, pulmonary arterial smooth muscle, and neutrophils.[3,8–11]

Nitric oxide – damaging versus protective actions

Although nitric oxide is a radical, it exhibits relatively low reactivity.[13] Indeed, a large body of literature suggests that NO is protective against injury and inflammation. Wink *et al.*[14] reported that NO can exhibit antioxidant properties. A number of studies have reported that NO can react with alkoxyl and peroxyl radical intermediates to terminate oxidant-induced lipid radical chain propagation reactions and, thus, inhibit free radical-mediated lipid peroxidation.[15–18] NO has also been shown to inhibit superoxide anion production by neutrophils by inhibition of NADPH oxidase.[19] In addition, NO can decrease the recruitment of neutrophils by reducing neutrophil deformity, decreasing the production of endothelial adhesion molecules, and decreasing the production of interleukin-8, a chemokine, by lung epithelial cells.[20–24] Inhibition of nuclear factor-kappa B (NF-κB) activation in response to inflammatory stimuli, such as silica or lipopolysaccharide, has also been reported.[25–28] Reported mechanisms for this NO-induced inhibition of NF-κB binding to DNA have included nitrosation of cysteine residues on the p50 subunit of NF-κB and inhibition of IκBα degradation, which maintains NF-κB in its inactive state.[29–31] Since NF-κB is a transcription factor for a variety of inflammatory cytokines, chemokines and growth factors, this property of NO could be protective against the pathogenic effects of silica or lipopolysaccharide.

Although NO exhibits low reactivity, it can combine with superoxide anion, $O_2^{\bullet-}$, to form peroxynitrite, $ONOO^-$.[32,33] The reaction of NO and $O_2^{\bullet-}$ is irreversible and occurs at a near diffusion-limited rate.[13] In fact, NO can out-compete superoxide dismutase for $O_2^{\bullet-}$. Peroxynitrite is a potent oxidant.[13] It reacts with tyrosine residues to produce nitrotyrosine.[13,34,35] Such nitrosation has been associated with disruption of actin filaments in the cytoskeleton, inhibition of mitochondrial enzymes, oxidation of surfactant protein A, and oxidative depletion of plasma sulfydryls and antioxidants.[13,36,37] Oxidant damage resulting from peroxynitrite has been linked to enhanced lipid peroxidation, DNA damage, inactivation of enzymes and proteins, increased proliferation, and tumor promotion.[18,38,39] In addition, NO has been proposed to exhibit a pro-inflammatory action by enhancing NF-κB activation in response to inflammatory agents.[40,41] Such activation of NF-κB has been linked in NO-induced enhancement of the production of tumor necrosis factor alpha (TNF-α) and interferon-γ (IFN-γ).[42] Lastly, NO generators have been shown to stimulate prostaglandin E_2 (PGE_2) production by rat alveolar macrophages and lung fibroblasts *in vitro*.[43]

Goal of this review

From the above discussion, it is clear that NO has been proposed to exhibit both oxidant/damaging/inflammatory and antioxidant/anti-inflammatory actions. This manuscript will attempt to clarify this issue by evaluating the pulmonary responses to four pathogenic agents – asbestos, silica, ozone, and lipopolysaccharide. Evidence will be presented that iNOS is induced and NO production enhanced in lung cells exposed to these agents. Lastly, the role of this induced NO production in the pathogenesis of lung disease resulting from exposure to each of these agents will be evaluated.

ASBESTOS

Asbestos refers to a group of naturally occurring fibrous silicate minerals. Mineralogically, asbestos fibers are classified as serpentines and amphiboles. Chrysotile is a serpentine asbestos, while crocidolite, actinolite, tremolite, amosite and anthophyllite are amphiboles. Inhalation of asbestos is associated with asbestosis (interstitial fibrosis), lung cancer, pleural fibrosis, and mesothelioma.[44] Free radicals generated directly from asbestos fibers as well as oxidants generated as a result of cellular interaction with fibers have been linked to DNA damage, inflammation, and proliferation associated with the pathogenesis of asbestos-induced diseases.[45] The following is a summary of evidence that nitric oxide is generated in response to asbestos exposure and a discussion of the role of nitric oxide in asbestos-induced diseases.

Nitric oxide production in response to in vitro *exposure to asbestos*

Thomas *et al.*[46] reported that exposure of rat alveolar macrophages to asbestos fibers (10 μg/ml) resulted in a significant increase in nitric oxide release measured 48 h after exposure. In their test system, chrysotile was a more potent stimulant than crocidolite (on an equal mass basis). Chrysotile-induced nitric oxide production by rat alveolar macrophages 24 h after *in vitro* exposure was also reported by Iguchi *et al.*[47] who found crocidolite and amosite to be inactive at a 150 μg/ml dose. *In vitro*, a 24-h exposure of rat alveolar macrophages or RAW 264.7 mouse peritoneal macrophages to crocidolite (10 $\mu g/cm^2$) resulted in induction of mRNA for iNOS and activation of the iNOS promoter gene.[48] Treatment of a mouse alveolar macrophage cell line (MH-S) with crocidolite (25 $\mu g/cm^2$) increased NOS activity and NO production by ~4-fold at the 24-h exposure time point.[49] Crocidolite (6 $\mu g/cm^2$ for 24 h) also stimulated mRNA levels for iNOS and NO production in an alveolar type II epithelial cell line (A549).[50] Treatment of these cells with aminoguanidine, an iNOS inhibitor, not only decreased asbestos-induced NO production but also inhibited 8-OHdG formation, suggesting a link between NO production and asbestos-induced DNA damage. In contrast, exposure of rat parietal pleural mesothelial cells to crocidolite or chrysotile failed to induce NO production. However, asbestos-induced mRNA for iNOS and NO production was noted if interleukin-1 (IL-1) was present in the culture medium.[51]

Nitric oxide production in response to in vivo *exposure to asbestos*

Intratracheal instillation of rats with asbestos has been shown to increase NOS activity of lung tissue 48-h post-exposure.[47] On an equal mass basis, chrysotile was more potent than amosite. Dorger *et al.*[52] reported induction of mRNA for iNOS in lung tissue and increased immunohistochemical staining for iNOS protein and nitrotyrosine residues in bronchial and alveolar epithelial cells and alveolar macrophages 24 h after intratracheal instillation of mice with crocidolite (2 mg/kg). Similar results were reported in rats 24 h after intratracheal instillation of crocidolite (5 mg/kg), with increased iNOS protein and mRNA noted in lung tissue and positive staining for iNOS and nitrotyrosine in alveolar macrophages, alveolar epithelial cells and vascular endothelium.[53] Induction of NO has also been observed after inhalation exposure of rats to asbestos. A 6 h/day exposure to crocidolite or chrysotile asbestos (7–10 mg/m^3) for 3 days resulted in a greater than 2-fold increase in NO production from alveolar macrophages.[48] This NO production was temporally correlated with inflammation, measured by neutrophil infiltration. One week after inhalation exposure of rats (7–8 mg/m^3, 6 h/day, 5 days/week) to crocidolite or chrysotile, iNOS protein was elevated in alveolar macrophages resulting in a 2-3-fold increase in NO production by these phagocytes. Increased nitrotyrosine staining of alveolar macrophages, bronchiolar epithelial cells and alveolar bifurcation epithelial cells indicated that this enhanced NO production resulted in peroxynitrite formation and nitrosation-induced damage.[54]

Influence of nitric oxide on the pulmonary response to asbestos

The evidence above indicates that asbestos exposure either *in vitro* or *in vivo* can induce NO production by alveolar macrophages and alveolar epithelial cells. In some cases, an association was noted between the potency of different forms of asbestos to induce NO production and to cause pulmonary damage.[47] In other

Table 2. Response of rats and hamsters to asbestos exposure

Species	NO induction	Pulmonary responses	
Rat	↑ mRNA for iNOS (lung tissue)	↓ Gas exchange	53%
	↑ iNOS protein (lung tissue)	↑ Edema	32%
	↑ iNOS staining (lung sections)	↑ BALF protein	857%
	↑ Nitrotyrosine staining (lung sections)	↑ BAL neutrophils	278%
		↑ Lung MPO	438%
Hamster	No evidence of induction of iNOS protein, message, or reactive product (peroxynitrite)	↓ Gas exchange	23%
		↑ Edema	1%
		↑ BALF protein	43%
		↑ BAL neutrophils	0%
		↑ Lung MPO	50%

Crocidolite exposure – intratracheal instillation of crocidolite (5 mg/kg body weight): pulmonary responses evaluated 24 h post-exposure. Modified from Dorger *et al.*[53]

cases, a temporal association between NO production and pulmonary inflammation was noted.[48] The presence of asbestos-induced nitrotyrosine staining in lung tissue has also been taken to indicate a role of NO in asbestos-induced lung injury.[52–54] Further correlation between NO production and asbestos-induced inflammation and injury has been reported recently by Dorger *et al.*[53] This study compared the acute pulmonary reaction of rats and hamsters to intratracheal instillation of crocidolite (5 mg/kg). The rat/hamster comparison was investigated because rat alveolar macrophages exhibit iNOS induction and enhanced NO release in response to LPS, IFN-γ or TNF-α, while hamster alveolar macrophages do not.[55,56] As shown in Table 2, 1 day after intratracheal instillation of crocidolite the rat showed the following indications of induction of NO production: (i) an increase in mRNA for iNOS in lung tissue measured by Northern blot; (ii) an increase in lung iNOS protein measured by Western blot; (iii) an increase in immunohistochemical staining for iNOS in lung slices; and (iv) an increase in nitrotyrosine staining of lung slices. In contrast, lung tissue from asbestos-exposed hamsters showed no indication of NO induction. Correlating with this NO induction, rat lungs exhibited substantial evidence of alveolar infiltration of neutrophils, damage to the alveolar air/blood barrier, edema and activation of myeloperoxidase (MPO) in lung tissue, while hamsters exhibited a much less pronounced degree of inflammation or damage in response to asbestos (Table 2). These quantitative indicators of pulmonary response were supported by histopathology results, where hemorrhage, alveolar inflammatory cells, and thickening of the alveolar septa were more dramatic in asbestos-exposed rat lungs than the hamster.

The role of NO in asbestos-induced injury was also investigated using iNOS knockout mice.[52] In this model, both protective and damaging effects of NO were observed (Table 3). iNOS knockout mice exhibited greater inflammation, measured as neutrophil harvested by bronchoalveolar lavage, histological evidence of inflammatory cell infiltration, lung mRNA for TNF-α, and TNF-α levels in bronchoalveolar lavage fluid. These results suggest a protective role of NO against asbestos-induced inflammation. In contrast, asbestos-exposed iNOS knockout mice exhibited significantly lower lung damage, measured as lavage fluid protein, lavage fluid

Table 3. Role of nitric oxide in the pulmonary response to asbestos

Role of NO	Model	Pulmonary response (Response of iNOS knockout compared to wild-type mice)	
Protective	iNOS KO	↑ BAL neutrophils	26%
		↑ Lung MPO	76%
		↑ Lung mRNA for TNF-α	
		↑ BALF TNF-α	53%
		↑ Histological evidence of inflammatory cells	
Damaging	iNOS KO	↓ BALF protein	24%
		↓ BALF LDH	51%
		↓ Histological evidence of alveolar septal thickening	

Crocidolite exposure of mice – intratracheal instillation of crocidolite (2 mg/kg body weight): pulmonary responses evaluated 24 h post-exposure. Modified from Dorger *et al.*[52]

lactate dehydrogenase (LDH) activity, and histological evidence for thickening of the alveolar septa. These results suggest a damaging role of NO in asbestos-induced lung injury.

Conclusion

There is strong evidence from both *in vitro* and *in vivo* exposure models that asbestos induces iNOS protein synthesis and the production of NO by alveolar macrophages and alveolar epithelial cells. Evidence indicates that NO plays a significant role in acute asbestos-induced lung injury probably by peroxynitrite-dependent nitrosation of alveolar tissue. However, evidence also indicates that NO is protective against the acute inflammatory response to asbestos. The role of NO in chronic pathogenesis in response to asbestos has not been investigated.

SILICA

Inhalation of crystalline silica (SiO_2) can result in silicosis. Acute silicosis results from exposures to relatively high levels of silica and is characterized by the rapid development of pulmonary edema, interstitial inflammation and alveolar lipoproteinosis. Patients often suffer from labored breathing, fatigue, cough, weight loss, and compromised gas exchange. Chronic silicosis develops over decades and is characterized by fibrotic nodular lesions. As fibrosis progresses, restrictive lung disease and decreased gas exchange can become marked.[57] Silica exposure has also been associated with lung cancer.[58] Free radicals generated from silica particles and reactive species produced by lung cells exposed to silica have been linked to silica-induced fibrosis and lung cancer.[59,60] The following is a summary of the evidence that NO is generated upon silica exposure and a discussion of the role of NO in silica-induced lung disease.

Nitric oxide production in response to in vitro *exposure to silica*

Srivastava *et al.*[61] have reported that the exposure of a mouse macrophage cell line (IC-21) to silica (10 μg/ml) resulted in a greater than 12-fold increase in NO after 4 h of exposure. In contrast, silica exposure of a mouse peritoneal macrophage cell line (RAW 264.7) caused a small, but not significant, induction of the iNOS gene promoter.[48] *In vitro* exposure of primary rat alveolar macrophages to silica (5–100 μg/ml) failed to induce NO production after a 24-h treatment.[62] Primary rat alveolar type II epithelial cells were also shown to be non-responsive to silica exposure with respect to NO production.[62] Huffman *et al.*[9] have shown that alveolar macrophages will produce NO in response to *in vitro* treatment with silica if the cells were incubated in conditioned-medium from bronchoalveolar cells harvested from silica-exposed rats. Therefore, intercellular mediators appear to be critical to the responsiveness of cells to silica. This requirement for priming is unique to NO and is not required for silica-induced stimulation of TNF-α, MIP-2, IL-6, or IL-1β production.[62]

Nitric oxide production in response to in vivo *exposure to silica*

In contrast to *in vitro* studies, *in vivo* exposure of rats to silica has been consistently associated with induction of NO. Intratracheal instillation of rats with crystalline silica (10 mg/100 g body weight) resulted in a 5-fold increase in NO production from bronchoalveolar lavage cells (BALC), a 3-fold increase in mRNA for iNOS in BALC, and a 5-fold increase in zymosan-stimulated NO-dependent chemiluminescence from alveolar macrophages harvested 24-h post-exposure.[9,63] Similarly, induced iNOS levels were reported in alveolar macrophages in response to inhalation of crystalline silica (250 mg/m^3, 5 h/day, for 10 days). This induction was evident 1 week after exposure.[61] Porter *et al.*[64] conducted a 116-day inhalation exposure study with crystalline silica (15 mg/m^3, 6 h/day, 5 days/week). Nitrate and nitrite (NOx) was elevated by 1.8-fold after 10 days of exposure, while NO-dependent chemiluminescence was elevated 15-fold at this exposure time. This level of induction remained relatively constant for the first 41 days of exposure, increasing explosively thereafter (a 22-fold increase in BALF NOx and a 151-fold increase in NO-dependent chemiluminescence after 116 days of exposure). This NO production was associated with immunohistochemical evidence of iNOS induction and nitrotyrosine residues in lung sections at 79 and 116 days of exposure. Alveolar macrophages and alveolar type II epithelial cells were identified by *in situ* immunohistochemistry as the major sources of i-NOS-derived NO in silica-exposed rat lungs.

Influence of nitric oxide on the pulmonary response to silica

A number of studies have reported an association between silica-induced NO production and pathogenesis. Blackford *et al.*[65] reported that, when normalized to an equivalent exposure by particle number, the sequence of potency for four dust particles (silica > coal >> carbonyl iron > titanium dioxide) in the induction of mRNA for iNOS in bronchoalveolar lavage cells harvested from rats 24 h after an

Table 4. Role of nitric oxide in the pulmonary response to silica

Role of NO	Model	Pulmonary response (Response of iNOS knockout compared to wild-type mice)		Reference
Damaging	iNOS KO	↓ # of histopathological lesions		61
		1 week post	80%	
		6 weeks post	91%	
		12 weeks post	95%	
		↓ Size of histopathological lesions		
		1 week post	60%	
		6 weeks post	93%	
		12 weeks post	95%	
Damaging	iNOS KO	↓ BALF LDH	20%	67
		↓ BAL albumin	23%	
		↓ AM – chemiluminescence	65%	
		↓ BALF TNF-α	44%	
		↓ Lung hydroxyproline	86%	
		↓ Alveolitis (histopathology)	52%	
		↓ Lipoproteinosis (histopathology)	60%	

Srivastava *et al*.[61] – exposure by inhalation of silica (250 mg/m^3, 5 h/day, 10 days).
Zeidler[67] exposure by pharyngeal aspiration (40 mg/kg body weight), 42 days post-exposure.

intratracheal instillation of dust (5 mg/100g body weight) was similar to the potency sequence for lung damage (BALF protein) and inflammation (BAL neutrophils) with these dusts. Castranova *et al*.[66] evaluated bronchoalveolar lavage cells from a control volunteer, a coal miner exposed to high silica levels but with a normal chest radiograph, and a coal miner exposed to high silica levels with an abnormal chest radiograph. Both the sequence for mRNA levels for iNOS and for NO-dependent chemiluminescence followed disease progression, *i.e*. miner abnormal X-ray > miner normal X-ray > control. A study by Porter *et al*.[64] reported a temporal association between NOx in bronchoalveolar lavage fluid and markers of pulmonary inflammation and damage and an anatomical association between immunohistochemical staining of tissue sections for iNOS and nitrotyrosine and areas of granulomas in the lung and bronchial associated lymph nodes, resulting from inhalation of silica in a rat model.

A more direct link between silica-induced NO production and pathogenesis was obtained using iNOS knockout mice. These results are summarized in Table 4. Srivastava *et al*.[61] reported that silica-induced pulmonary lesions were strikingly reduced in both number and size in iNOS knockout mice compared to wild-type mice evaluated histologically 1–12 weeks' post-exposure. Zeidler[67] reported a similar diminished response of iNOS knockout mice 42 days after intratracheal silica exposure. This protection was statistically significant for a number of pulmonary markers of damage and macrophage activation as well as for histological scores for alveolitis and lipoproteinosis. Interestingly, iNOS knockout provided protection in a subchronic exposure (42 days after silica exposure) but not in an acute exposure (1 day after silica exposure).[67] This suggests that the role of NO is more evident in longer term pathogenic events.

Conclusion

Although silica is not an effective stimulant of NO using *in vitro* alveolar macrophage or alveolar type II epithelial cell systems, crystalline silica is a potent stimulant of iNOS induction and NO production *in vivo*. This suggests the intercellular communication is important. Both associative and direct evidence support the hypothesis that NO plays a damaging role, most likely through peroxynitrite, in initiation of lung injury and progress of silica-related disease.

OZONE

Ozone (O_3) in ambient air is formed from nitrogen oxides and volatile organic compounds in the presence of sunlight. Ozone is also generated occupationally from electrical arcing of equipment, such as welding. Acute exposure to ozone is associated with airway hyper-reactivity, inflammation and tissue damage, leading to the exacerbation of asthma and bronchitis. Repeated ozone exposure can lead to obstructive lung disease and fibrosis.[68] The following is a summary of evidence that NO is generated in response to ozone exposure and a discussion of the role of NO in ozone-induced lung injury.

Table 5. Role of nitric oxide in the pulmonary response to ozone

Role of NO	Model	Pulmonary response (Response of iNOS knockout compared to wild-type mice)		Reference
Protective	iNOS KO (1 ppm O_3, 8 h/night, for 3 nights)	↑ BALF protein	40%	72
		↑ BAL neutrophils	1215%	
		↑ BALF MIP-2	285%	
		↑ BALF MMP-9	229%	
Damaging	iNOS KO (0.8 ppm for 3 h, evaluated 24 h post-exposure	↓ BALF protein	100%	69
		↓ nitrotyrosine staining of lung sections		
Damaging	iNOS KO (0.8 ppm for 3 h, evaluated 24 h post-exposure	↓ BALF protein	32%	74
		↓ Bronchoalveolar lavage cell number	35%	
		↓ PGE_2 from alveolar macrophages	100%	
		↓ Nitrotyrosine staining of lung sections		

Nitric oxide production in response to in vitro *exposure to ozone*

The database for the effects of *in vitro* exposure to ozone on induction of NO is sparse. A 1-h exposure of primary rat alveolar macrophages or rat tracheal epithelial cells to 0.5 ppm ozone failed to induce mRNA levels for iNOS 3 h post-exposure (Dr Murali Rao, NIOSH, personal communications). However, whether iNOS message would be induced in response to a higher level or longer duration of ozone exposure is yet to be tested.

Nitric oxide production in response to in vivo *exposure to ozone*

Exposure of mice to ozone (0.8 ppm for 3 h) has been shown to induce NO production in alveolar macrophages harvested by bronchoalveolar lavage 24-h post-exposure.[69] This induction was evidenced as follows: (i) increased mRNA for iNOS in alveolar macrophages; (ii) increased iNOS protein in alveolar macrophages; and (iii) increased NO and peroxynitrite production by alveolar macrophages. Similar induction of NO has also been reported in rat models. Exposure to ozone (1 ppm for 3 h) increased NO production from bronchoalveolar lavage cells by 1.7-fold and immunohistochemical staining of lung sections for iNOS in alveolar macrophages and alveolar type II epithelial cells 2 days post-exposure.[70] These ozone-induced responses are more pronounced 1 day following exposure to 2 ppm ozone for 3 h, that is, induction of iNOS protein in alveolar macrophages and type II cells isolated from ozone-exposed rats resulted in a 3.6-fold increase in NO production by alveolar macrophages and a 14.3-fold increase in NO production from alveolar type II epithelial cells.[71] This induction of iNOS protein in response to ozone is associated with an activation of NF-κB. Inhibition of NF-κB with pyprolidine dithiocarbamate effectively inhibited the ozone-induced increase in iNOS protein in this model system.[71]

Influence of nitric oxide on the pulmonary response to ozone

The results above indicate that exposure to ozone can increase mRNA for iNOS, iNOS protein and NO production. Several

Table 6. Damaging role of nitric oxide in the pulmonary response to LPS

Exposure	Model	Pulmonary response (Response of test animals, NOS inhibitor or KO, compared to untreated or wild-type)		Reference
i.v. exposure of rabbits to LPS (5 mg/kg)	i.v. treatment with aminoguanidine	↓ BALF albumin	69%	86
		↓ BAL neutrophils	56%	
		↓ Histological score for lung injury	50%	
i.p. exposure of mice to LPS (25 mg/kg)	iNOS KO	↓ BALF LDH	100%	85
		↓ Edema	100%	
		↓ Histological score for lung injury	30%	

investigations have been conducted to evaluate the role of this elevated NO production in ozone-induced lung damage and inflammation. Kenyon *et al.*[72] reported that NO had a protective effect and mitigated ozone-induced pulmonary responses. This conclusion is based on the fact that ozone-induced inflammation and damage were greater in iNOS knockout mice than wild-type mice (Table 5). Support for a protective role of NO against the adverse effects of oxidant exposure also comes from a study by Kobayashi *et al.*[73] These investigators reported that markers of lung damage and inflammation are higher in iNOS KO mice exposed to hyperoxia than wild-type mice.

In contrast, several studies conclude that NO is damaging and enhances the adverse pulmonary effects of ozone. Ozone (0.8 ppm for 3 h) results in nitrotyrosine staining of mouse lung sections 24 h post-exposure, indicating peroxynitrite-induced lung damage had occurred.[69,74] Pretreatment of rats with a peroxynitrite scavenger, ebselen (10 mg/kg body weight intraperitoneally 1 h before and 7 h after O_3 exposure), not only decreased ozone-induced (2 ppm O_3 for 4 h) nitrotyrosine staining of alveolar macrophages in lung sections 18 h post-exposure, but also decreased ozone-induced lung damage (BALF albumin) by 78% and inflammation (BAL neutrophils) by 37%.[75] Alveolar macrophage-derived NO appears to play an important role in pulmonary responses to ozone.[76] Pretreatment of rats with gadelinium chloride (7 mg/kg body weight intravenously 24 h before exposure to 2 ppm O_3 for 3 h) decreased iNOS staining of alveolar macrophages but not alveolar type II cells 4 h post-exposure. This decrease in macrophage iNOS resulted in a 75% decline in NO levels in BALF. Inhibition of NO production by alveolar macrophage was associated with a 19% decrease in BALF protein and a 12% decrease in BAL neutrophils in $GaCl_3$-pretreated, ozone-exposed rats. More direct evidence of an adverse role on NO in ozone-induced lung damage involves studies with iNOS knockout mice (Table 5). These studies report that BALF protein, BAL cell number, lung nitrotyrosine staining and PGE_2 production by alveolar macrophages in response to ozone exposure were mitigated in iNOS knockout mice compared to wild-type mice.[69,74]

Conclusion

Evidence indicates that exposure to ozone increases NO production by alveolar macrophages and alveolar type II epithelial cells. Nitrotyrosine staining of ozone-exposed lung tissue indicates peroxynitrite is formed as a result of this enhanced NO production and results in NO-dependent lung injury. Although there is a report to the contrary, most information supports an adverse role for NO in ozone-induced lung injury.

LIPOPOLYSACCHARIDE

Organic dusts found in grain-handling operations, sewage treatment plants, animal confinement facilities, cotton textile plants, and wood-processing facilities can be contaminated with endotoxin (a product of the cell wall of Gram-negative bacteria). The bioactive component of endotoxin is lipopolysaccharide (LPS).[77] Occupational exposure to dusts containing LPS can result in an acute response termed organic dust toxic syndrome, which is characterized by fever, headache, chest tightness and leukocytosis.[78] Chronic exposure to LPS can result in obstructive lung disease.[78]

Nitric oxide production in response to in vitro *exposure to LPS*

In vitro exposure of primary rat alveolar macrophages to LPS (0.1–5 μg/ml) has been reported to increase mRNA for iNOS and iNOS protein 24 h post-exposure, while increasing NO production by alveolar macrophages by 29–50-fold.[62,79,80] LPS (10 μg/ml) has also been reported to induce NO production from an alveolar type II cell line.[8] Kanj *et al.*[62] compared the responsiveness of primary rat alveolar macrophages with that of primary type II cells and a rat alveolar epithelial cell line (RLE-6TN) and found NO production in response to LPS to be 5.8-fold higher in primary alveolar macrophages than primary type II cells, with the alveolar epithelial cell line being significantly less responsive than primary type II cells.

Nitric oxide production in response to in vivo *exposure to LPS*

Numerous studies indicate that LPS is a potent stimulant of iNOS activity in the lung. Intratracheal instillation of rats with LPS (250 μg/100 g body weight) resulted in significant induction of NO 24 h post-exposure: (i) mRNA for iNOS in lung tissue increased 4-fold; (ii) mRNA for iNOS in bronchoalveolar lavage cells increased 7-fold; and (iii) NO-dependent chemiluminescence from alveolar macrophages increased 9-fold.[63] Similar up-regulation of NO production was reported 18 h after inhalation exposure of rats to endotoxin (2.2×10^4 EU/m^3 for 3 h): (i) NO products (nitrate and nitrite) increased in BALF by 1.7-fold; (ii) NO production from BAL cells increased 1.9-fold; (iii) mRNA for iNOS in BAL cells increased 11.5-fold; and (iv) NO-dependent chemiluminescence from alveolar macrophages increased 33-fold.[80] Greenberg *et al.*[81] reported the increase in NO production and iNOS levels 4 h after intratracheal instillation of LPS in mice was demonstra-

Table 7. Protective role of nitric oxide in the pulmonary response to LPS

Exposure	Model	Pulmonary response (Response of test animals, NOS inhibitor or KO, compared to untreated or wild-type)		Reference
IT exposure of mice to endotoxin (40 μg/mouse), 6 h post-exposure	i.p. treatment with L-NAME (13 mg/kg)	↑ mRNA for IL-6 (lung) ↑ IL-6 in lung tissue ↑ mRNA for TNF-α (lung) ↑ TNF-α in lung tissue	300% 65% 26% 66%	27
i.v. exposure of rabbits to LPS (5 mg/kg), 6 h post-exposure	Inhaled NO (10 ppm)	↓ BALF albumin ↓ BAL neutrophils ↓ Chemiluminescence by BAL cells ↓ NF-κB activity of alveolar macrophages	100% 100% 51% 100%	83
i.v. exposure of rabbits to LPS (5 mg/kg), 6 h post-exposure	Inhaled NO (10 ppm)	↓ BALF protein ↓ BAL LDH ↓ BAL neutrophils ↓ Chemiluminescence by alveolar macrophages ↓ NF-κB activity of alveolar macrophages ↓ IL-1 production by alveolar macrophages ↓ IL-8 production by alveolar macrophages	53% 93% 100% 100% 100% 41% 100%	87
Pharyngeal aspiration of mice with 1.2 mg/kg LPS plus 5000 U/mouse IFN-γ, 72 h post-exposure	iNOS KO	↑ BALF LDH ↑ BALF albumin ↑ BALF neutrophils ↑ Chemiluminescence by alveolar macrophages ↑ BALF TNF-α ↑ BALF MIP-2	54% 136% 23% 90% 72% 127%	67
IT exposure of mice to LPS, 4 h post-exposure	i.p. treatment with L-NIL (10 mg/kg)	↑ BAL neutrophils		81
IT exposure of mice to LPS, 4 h post-exposure	iNOS KO	↑ BAL neutrophils		81
Pulmonary exposure to LPS	L-Arginine treatment	↓ IL-1β by alveolar macrophages ↓ TNF-α by alveolar macrophages		88

ble in pulmonary neutrophils and alveolar macrophages. Intravenous exposure of an isolated-perfused rat lung to LPS resulted in a 5-fold induction of NOS activity in the lung.[82] Similarly, intravenous treatment of rabbits with LPS (5 mg/kg body weight) has been shown to increase NO product levels in BALF and NO production by BAL cells.[83] Intravenous LPS also increased mRNA for iNOS with induction in alveolar macrophages being greater than alveolar type II cells which was greater than interstitial macrophages.[10] Intraperitoneal injection of LPS has also been shown to induce iNOS in both mouse and rat models.[84,85] Pulmonary responses include: (i) increased mRNA for iNOS in lung tissue; (ii) increased NOS activity in lung tissue; (iii) increase iNOS protein in lung tissue; and (iv) increased nitrotyrosine staining in airway epithelium, alveolar epithelial cells, alveolar macrophages, and interstitial macrophages.

Influence of nitric oxide on the pulmonary response to LPS

The studies described above clearly demonstrate that LPS-induced pulmonary inflammation is associated with induction of iNOS and increased production of NO by pulmonary macrophages and epithelial cells. However, conflicting studies exist concerning the role of NO in LPS-induced pulmonary responses. Evidence that LPS induces nitrotyrosine staining of lung tissue suggests a damaging role of NO.[85] However, inhibition of LPS-induced NO production by peritoneal macrophages *in vitro* with the NOS inhibitor, L-NAME, results in enhanced NF-κB–DNA binding.[27] Therefore, the authors suggested that NO acts to attenuate LPS-induced cytokine production and inflammation.

In vivo studies suggesting that NO enhances LPS-induced pulmonary inflammation and damage are summarized in

Table 6. Treatment of rabbits with the iNOS inhibitor, aminoguanidine, attenuated lung injury and inflammation following intravenous exposure to LPS (5 mg/kg body weight).[86] Similarly, LPS-induced damage 12 h following intraperitoneal injection of LPS (25 mg/kg body weight) was significantly lower in iNOS knockout mice compared to wild-type mice.[85]

In contrast, several studies report that NO plays a protective role in suppressing pulmonary inflammation and damage resulting from LPS exposure (Table 7). Inhalation of NO or increasing the endogenous production of NO via NOS by supplementation of L-arginine has been reported to decrease markers of lung damage (BALF protein, albumin and LDH), decrease infiltration of neutrophils into the alveolar space, and attenuate the activation of alveolar macrophages (activation of NF-κB, production of cytokines, and generation of reactive oxidant species).[83,87,88] Inhibition of NOS with L-NAME or L-NIL augmented the adverse pulmonary effects of LPS exposure.[27,81] Similarly, knockout of the iNOS gene resulted in enhanced neutrophil infiltration, lung damage, cytokine production and oxidant generation by alveolar macrophages after LPS + IFN-γ exposure.[27,67] It is of interest that the protective effects of NO seen in my laboratory were apparent 72 h post-exposure but not 24 h post exposure.[67]

Conclusion

Although both damaging and protective effects of NO on LPS-induced pulmonary responses have been reported, the majority of studies suggest that NO acts to mitigate the adverse pulmonary responses to LPS exposure.

SUMMARY

In vitro studies present conflicting evidence as to whether NO expresses oxidant/damaging/inflammatory or antioxidant/protective/anti-inflammatory properties. Some of this controversy may depend on the ratio of NO versus peroxynitrite generated in the test system investigated, *i.e.* NO expressing protective effects in low $O_2^{\bullet-}$ conditions while being damaging in the presence of high $O_2^{\bullet-}$. The action of NO may also depend on the set point of signaling pathways at the time of study. For example, NO has been reported to inhibit silica-induced activation of NF-κB in serum-starved RAW 264.7 macrophages where basal NF-κB activity is low but potentiate NF-κB activation when silica exposure of RAW 264.7 cells was conducted in serum-containing medium where basal NF-κB is somewhat activated. *In vivo*, studies consistently suggest a damaging role for NO in the pathogenesis of silicosis, while most data suggest that NO is anti-inflammatory after exposure to lipopolysaccharide. This discrepancy may reflect differences in signaling pathways for silica versus lipopolysaccharide, *i.e.* silica interacts with scavenger receptors while LPS act through Toll-like receptors. Additionally, the opposing roles of NO in the pathogenesis of pulmonary disease in response to silica versus LPS may be related to the amount of peroxynitrite generated. Silica has been shown to induce directly $O_2^{\bullet-}$ production by alveolar macrophages, while LPS is not a direct stimulant but rather potentiates $O_2^{\bullet-}$ release from alveolar macrophages in response to a second stimulus. Therefore, the peroxynitrite:NO ratio may be higher in silica-exposed lungs compared to LPS-exposed lungs. It is clear that further investigation is required to understand fully the role of nitric oxide and/or peroxynitrite in the initiation and progression of pulmonary injury, inflammation, and disease resulting from exposure to various environmental and occupational agents.

REFERENCES

1. Lancaster Jr JR. Nitric oxide in cells. *Am Sci* 1992; **80**: 248–259.
2. Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; **43**: 109–141.
3. Schmidt HHHW, Seifert R, Bohem E. Formation and release of nitric oxide from human neutrophils and HL-60 cells induced by a chemotactic peptide, platelet activation factor, and leukotriene B4. *FEBS Lett* 1989; **244**: 357–360.
4. Miles PR, Bowman L, Rengasamy A, Huffman L. Constitutive nitric oxide production by rat alveolar macrophages. *Am J Physiol* 1998; **274**: L360–L368.
5. Miles PR, Bowman L, Rengasamy A, Huffman LJ. Alveolar type II cells cNOS activity and ATP levels are increased by lung surfactant or DPPC vesicles. *Am J Physiol* 1997; **273**: L339–L346.
6. Dinh-Xuan AT, Higenbottam TW, Chelland CA *et al*. Impairment of endothelium-dependent pulmonary artery relaxation in chronic obstructive lung disease. *N Engl J Med* 1991; **324**: 1539–1547.
7. Belvisi MG, Stetton CD, Yacoub M, Barnes PJ. Nitric oxide is the endogenous neurotransmitter of bronchodilator nerves in humans. *Eur J Pharmacol* 1992; **210**: 221–222.
8. Gutierrez HH, Pitt BR, Schwarz M *et al*. Pulmonary alveolar epithelial inducible NO synthase gene expression: regulation by inflammatory mediators. *Am J Physiol* 1995; **268**: L501–L508.
9. Huffman LJ, Judy DJ, Castranova V. Regulation of nitric oxide production by rat alveolar macrophages in response to silica exposure. *J Toxicol Environ Health,* Part A 1998; **53**: 29–46.
10. Wizemann TM, Gardner CR, Laskin JD *et al*. Production of nitric oxide and peroxynitrite in the lung during acute endotoxemia. *J Leukoc Biol* 1994; **56**: 759–768.
11. Jorens PG, VanOverveld FJ, Vermeire PH, Bult H, Herman AG. Synergism between interleukin-1β and interferon-γ, an inducer of nitric oxide synthase in rat lung fibroblasts. *Eur J Pharmacol* 1992; **224**: 7–12.
12. Nakayama DK, Geller DA, Lowenstein CJ *et al*. Cytokines and lipopolysaccharide induce nitric oxide synthase in cultured rat pulmonary arterial smooth muscle. *Am J Respir Cell Mol Biol* 1992; **7**: 471–476.
13. Beckman JS, Koppenol WH. Nitric oxide, superoxide and peroxynitrite: the good, the bad, and the ugly. *Am J Physiol* 1996; **271**: C1424–C1437.
14. Wink DA, Mitchell JB. Chemical biology of nitric oxide: insights into regulatory, cytotoxic and cytoprotective mechanisms of nitric oxide. *Free Radic Biol Med* 1998; **25**: 434–456.

15. Niziolek M, Kortowski, Girotti AW. Nitric oxide inhibition of free radical-mediated lipid peroxidation in photodynamically treated membranes and cells. *Free Radic Biol Med* 2003; **34**: 997–1005.
16. Kelley EE, Wagner BA, Buettner GR, Burns CP. Nitric oxide inhibits iron-induced lipid peroxidation in HL-60 cells. *Arch Biochem Biophys* 1999; **370**: 97–104.
17. Padmaja S, Huie RE. The reaction of nitric oxide with organic peroxyl radicals. *Biochem Biophys Res Commun* 1993; **195**: 539–544.
18. Rubbo H, Radi R, Trujillo M *et al*. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation; formation of novel nitrogen-containing oxidized lipid derivatives. *J Biol Chem* 1994; **269**: 26066–26075.
19. Clancy RM, Leszczynska-Piziak J, Abramson SB. Nitric oxide, an endothelial cell relaxation factor, inhibits neutrophil superoxide anion production via a direct action on the NADPH oxidase. *J Clin Invest* 1992; **90**: 1116–1121.
20. Sato Y, Walley KR, Klut ME *et al*. Nitric oxide reduces the sequestration of polymorphonuclear leukocytes in lung by changing deformability and CD18 expression. *Am J Respir Crit Care Med* 1999; **159**: 1469–1476.
21. Hickey MJ. Role of inducible nitric oxide synthase in the regulation of leukocyte recruitment. *Clin Sci* 2001; **100**: 1–12.
22. Hickey MJ, Kubes P. Role of nitric oxide in regulation of leukocyte-endothelial cell interactions. *Exp Physiol* 1997; **82**: 339–348.
23. Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airways. *Am J Respir Crit Care Med* 1994: **149**: 538–551.
24. Lin HC, Wang CH, Yu CT, Hwang KS, Kuo HP. Endogenous nitric oxide inhibits neutrophil adherence to lung epithelial cells to module interleukin-γ release. *Life Sci* 2001; **69**: 1333–1344.
25. Chen F, Kuhn DC, Sun S-C Gaydos LJ, Demers LM. Dependence and reversal of nitric oxide production on NF-κB in silica and lipopolysaccharide induced macrophages. *Biochem Biophys Res Commun* 1995; **214**: 839–846.
26. Park SK, Lin HC, Murphy S. Nitric oxide regulates nitric oxide synthase-2-gene expression by inhibiting NF-kappa B binding to DNA. *Biochem J* 1997; **322**: 609–613.
27. Walley KR, McDonald TE, Higashimoto Y, Hayashi S. Modulation of proinflammatory cytokines by nitric oxide in murine acute lung injury. *Am J Respir Crit Care Med* 1999; **160**: 698–704.
28. Thomassen MJ, Buhrow LT, Connors MJ, Kaneko FT, Erzurum SC, Kavura MS. Nitric oxide inhibits inflammatory cytokine production by human alveolar macrophages. *Am J Respir Cell Mol Biol* 1997; **17**: 279–283.
29. Matthews JR, Blotting CH, Panico M, Morris HR, Hay RT. Inhibition of NF-kappa B DNA binding by nitric oxide *Nucleic Acids Res* 1996; **24**: 2236–2242.
30. Peng HB, Libby P, Liao JK. Induction and stabilization of I kappa B alpha by nitric oxide mediates inhibition of NF-kappa B. *J Biol Chem* 1995; **270**: 14214–14219.
31. Raychaudhuri B, Dweik R, Connors MJ *et al*. Nitric oxide blocks nuclear factor-κB activation in alveolar macrophages. *Am J Respir Cell Mol Biol* 1999; **21**: 311–316.
32. Ischiropoulos H, Zhu L, Beckman JS. Peroxynitrite formation from macrophage-derived nitric oxide. *Arch Biochem Biophys* 1992; **298**: 446–451.
33. Blough NV, Safirrou OC. Reaction of superoxide with nitric oxide to form peroxynitrite in alkaline aqueous solution. *Inorg Chem* 1995; **24**: 3502–3504.
34. Beckman JS. Oxidative damage and tyrosine nitration from peroxynitrite. *Chem Res Toxicol* 1996; **9**: 836–844.
35. Van der Vliet A, Cross CE. Oxidants, nitrosants and the lung. *Am J Med* 2000; **103**: 398–421.
36. Zhu S, Manuel M, Tanaka S, Choe N, Kagen E, Matalon S. Contribution of reactive oxygen and nitrogen species to particulate-induced lung injury. *Environ Health Perspect* 1998; **106 (Suppl 5)**: 1157–1163.
37. Van der Vliet A, Smith D, O'Neill CA *et al*. Interactions of peroxynitrite and human plasma and its constituents: oxidative damage and antioxidant depletion. *Biochem J* 1994; **303**: 295–301.
38. Eiserich JP, Patel RP, O'Donnell VB. Pathophysiology of nitric oxide and related species: free radical reactions and modification of biomolecules. *Mol Aspects Med* 1998; 221–357.
39. Hofseth LJ, Hussain SP, Wogan GW, Harris CC. Nitric oxide in cancer and chemoprevention. *Free Radic Biol Med* 2003; **34**: 955–968.
40. Laroux FS, Pavlick KP, Hines IN *et al*. Role of nitric oxide in inflammation. *Acute Physiol Scand* 2001; **173**: 113–118.
41. Kang JL, Kuungeum L, Castranova V. Nitric oxide up-regulates DNA-binding activity of nuclear factor-κB in macrophages stimulated with silica and inflammatory stimulants. *Mol Cell Biochem* 2000; **215**: 1–9.
42. Yang S, Porter VA, Cornfield DN *et al*. Effects of oxidant stress on inflammation and survival of iNOS knockout mice after marrow transplantation. *Am J Physiol* 2001, **281**: L922–L930.
43. Castranova V. The role of nitric oxide in the progression of pneumoconiosis. *Biochemistry* (Moscow) 2004; 69: 32–37.
44. Brody AR. Asbestos. In: Roth RA. (ed) *Comprehensive Toxicology, Vol 8 Toxicology of the Respiratory System.* New York: Elsevier Sciences, 1997; 393–413.
45. Shukla A, Gulumian M, Hei T, Kamp D, Rahman Q, Mossman BT. Multiple roles of oxidants in the pathogenesis of asbestos-induced diseases. *Free Radic Biol Med* 2003; **34**: 1117–1129.
46. Thomas G, Ando T, Verma K, Kagan E. Asbestos fibers and interferon-γ up-regulate nitric oxide production in rat alveolar macrophages. *Am J Respir Cell Mol Biol* 1994; **11**: 707–715.
47. Iguchi H, Kojo S, Ikeda M. Nitric oxide (NO) synthase activity in the lung and NO synthase in alveolar macrophages of rats increased on exposure to asbestos. *J Appl Toxicol* 1996; **16**: 309–315.
48. Quinlan TR, BeruBe KA, Hacker MP *et al*. Mechanism of asbestos-induced nitric oxide production by rat alveolar macrophages in inhalation and in vitro models. *Free Radic Biol Med* 1998; **24**: 778–788.
49. Aldieri E, Ghigo D, Tomatis M *et al*. Iron inhibits the nitric oxide synthesis elicited by asbestos in murine macrophages. *Free Radic Biol Med* 2001; **31**: 412–417.
50. Chao C-C, Park S-H, Aust AE. Participation of nitric oxide and iron in the oxidation of DNA in asbestos-treated human lung epithelial cells. *Arch Biochem Biophys* 1996; **326**: 152–157.
51. Choe N, Tanaka S, Kagan E. Asbestos fibers and interleukin-1 upregulates the formation of reactive nitrogen species in rat pleural mesothelial cells. *Am J Respir Cell Mol Biol* 1998; **19**: 226–236.
52. Dorger M, Allmeling A-M, Kiefmann R, Schropp A, Krombach F. Dual role of inducible nitric oxide synthase in acute asbestos-induced lung injury. *Free Radic Biol Med* 2002; **33**: 419–501.
53. Dorger M, Allmeling A-M, Kiefmann R, Munzing S, Messmer K, Krombach F. Early inflammatory response to asbestos exposure in rat and hamster lungs: role of inducible nitric oxide synthase. *Toxicol Appl Pharmacol* 2002; **181**: 93–105.
54. Tanaka S, Choe N, Hemenway DR, Zhu S, Matalon S, Kagan E. Asbestos inhalation induces reactive nitrogen species and nitrotyrosine formation in the lungs and pleura of the rat. *J Clin Invest* 1998; **102**: 445–454.
55. Dorger M, Jesch NK, Rieder G *et al*. Species differences in NO formation by rat and hamster alveolar macrophages *in vitro*. *Am J*

Respir Cell Mol Biol 1997; **16**: 413–420.
56. Jesch NK, Dorger M, Enders G *et al.* Expression of inducible nitric oxide synthase and formation of nitric oxide by alveolar macrophages: An interspecies comparison. *Environ Health Perspect* 1997; **105 (Suppl 5)**: 1297–1300.
57. Castranova V, Vallyathan V. Silicosis and coal workers' pneumoconiosis. *Environ Health Perspect* 2000; **108 (Suppl 4)**: 675–684.
58. Weill H, McDonald JC. Exposure to crystalline silica and risk of lung cancer: the epidemiological evidence. *Thorax* 1996; **51**: 97–102.
59. Lapp NL, Castranova V. How silicosis and coal workers' pneumoconiosis develop – a cellular assessment. *Occup Med* 1993; **8**: 35–56.
60. Shi X, Castranova V, Halliwell B, Vallyathan V. Reactive oxygen species and silica-induced carcinogenesis. *J Toxicol Environ Health*, Part B 1998; **1**: 181–197.
61. Srivastava KD, Rom WN, Jagirdar J, Yie T-A, Gordon T, Tchou-Wong K-M. Critical role of interleukin-1β and nitric oxide synthase in silica-induced inflammation and apoptosis in mice. *Am J Respir Crit Care Med* 2002; **165**: 527—533.
62. Kanj R, Kang J, Castranova V. Comparison of the responsiveness of primary alveolar macrophages (AM), primary alveolar type II cells, and a rat alveolar type II cell line (RLE-6TN) to lipopolysaccharide (LPS) and silica exposure. *Exp Biol* 2002; **16**: 861–863.
63. Blackford JA, Antonini JM, Castranova V, Dey RD. Intratracheal instillation of silica up-regulates inducible nitric oxide production in alveolar macrophages and neutrophils. *Am J Respir Cell Mol Biol* 1994; **11**: 426–431.
64. Porter DW, Millecchia L, Robinson VA *et al.* Enhanced nitric oxide and reactive oxygen species production and damage after inhalation of silica. *Am J Physiol* 2001; **283**: L485–L493.
65. Blackford JA, Jones W, Dey RD, Castranova V. Comparison of inducible nitric oxide synthase gene expression and lung inflammation following intratracheal instillation of silica, coal, carbonyl iron or titanium dioxide. *J Toxicol Environ Health* 1997; **51**: 203–218.
66. Castranova V, Huffman LT, Judy DJ *et al.* Enhancement of nitric oxide production by pulmonary cells following silica exposure. *Environ Health Perspect* 1998; **106 (Suppl 5)**: 1165–1169.
67. Zeidler PC. *Particle-induced Pulmonary Inflammation and Fibrosis: Role of Inflammatory Mediators in the Initiation and Progression of Occupational Disease.* Dissertation, West Virginia University, 2003.
68. Van Bree L, Last JA. Ozone. In: Roth RA. (ed) *Comprehensive Toxicology, Vol 8 Toxicology of the Respiratory System.* New York: Elsevier Sciences, 1997; 265–274.
69. Laskin DL, Fakhrzadeh L, Laskin JD. Nitric oxide and peroxynitrite in ozone-induced lung injury. *Adv Exp Med Biol* 2001; **500**: 183–190.
70. Pendino KJ, Laskin JD, Shuler RL, Penjubi CJ, Laskin DL. Enhanced production of nitric oxide by rat alveolar macrophages after inhalation of a pulmonary irritant is associated with increased expression of nitric oxide synthase. *J Immunol* 1993; **151**: 7196–7205.
71. Laskin DL, Sunil V, Guo Y, Heck DE, Laskin JD. Increased nitric oxide synthase in the lung after ozone inhalation is associated with activation of NF-κB. *Environ Health Perspect* 1998; **106 (Suppl 5)**: 1175–1178.
72. Kenyon NJ, van der Vliet A, Schock BC, Okamoto T, McGrew GM, Last JA. Susceptibility to ozone-induced acute lung injury in iNOS deficient mice. *Am J Physiol* 2002; **282**: L540–L545.
73. Kobayashi H, Hataishi R, Mitsufiji H *et al.* Antiinflammatory properties of inducible nitric oxide synthase in acute hyperoxic lung injury. *Am J Respir Cell Mol Biol* 2001; **24**: 390–397.
74. Fakhrzadeh L, Laskin JD, Laskin DL. Deficiency in inducible nitric oxide synthase protects mice from ozone-induced lung inflammation and tissue injury. *Am J Respir Cell Mol Biol* 2002; **26**: 413–419.
75. Ishii Y, Hashimoto K, Hirano K *et al.* Ebselen decreases ozone-induced pulmonary inflammation in rats. *Lung* 2000; **178**: 225–234.
76. Pendino KJ, Meidhof TM, Heck DE, Laskin JD, Laskin DL. Inhibition of macrophages with gadolinium chloride abrogates ozone-induced pulmonary injury and inflammatory mediator production. *Am J Respir Cell Mol Biol* 1995; **13**: 125–132.
77. Burrell R. Bacterial endotoxin. In: Roth RA. (ed) *Comprehensive Toxicology, Vol 8 Toxicology of the Respiratory System. New York:* Elsevier Sciences, 1997; 611–628.
78. Rylander R. Organic dusts. In: Roth RA. (ed) *Comprehensive Toxicology, Vol 8 Toxicology of the Respiratory System.* New York: Elsevier Sciences, 1997; 415–424.
79. Ruetten H, Thiemerman C. Prevention of the expression of inducible nitric oxide by aminoguanidine or aminoethyl-isothiourea in macrophages in the rat. *Biochem Biophys Res Commun* 1996; **225**: 525–530.
80. Huffman LJ, Judy DJ, Robinson VA, Castranova V. Inhalation of cotton dust is associated with increases in nitric oxide production by rat bronchoalveolar lavage cells. *Inhal Toxicol* 1997; **9**: 567–579.
81. Greenberg SS, Ouyung J, Zhao X, Parrish C, Nelson S, Giles TD. Effects of ethanol on neutrophil recruitment and lung host defense in nitric oxide synthase I and nitric oxide synthase II knockout mice. *Alcohol Clin Exp Res* 1999; **23**: 1435–1445.
82. Griffiths MJD, Curzen NP, Michell JA, Evans TW. *In vivo* treatment with endotoxin increases rat vasculature contractility despite NOS induction. *Am J Respir Crit Care Med* 1997; **156**: 654–658.
83. Koh Y, Kang J-L, Park W *et al.* Inhaled nitric oxide down-regulates intrapulmonary nitric oxide production in lipopolysaccharide-induced acute lung injury. *Crit Care Med* 2001; **29**: 1169–1174.
84. Liu SF, Adcock IM, Old RW, Evans TW. Differential regulation of the constitutive and inducible nitric oxide synthase mRNA by lipopolysaccharide treatment *in vivo* in rat. *Crit Care Med* 1996; **24**: 1219–1225.
85. Kristof AS, Goldberg P, Laubach V, Hussain SNA. Role of inducible nitric oxide synthase in endotoxin-induced acute lung injury. *Am J Respir Crit Care Med* 1998; **158**: 1883–1889.
86. Mikawa K, Nishina K, Tamada M, Takao Y, Maekawa N, Obara H. Aminoguanidine attenuates endotoxin-induced acute lung injury in rabbits. *Crit Care Med* 1998; **26**: 905–911.
87. Kang J-L, Pack W, Pack IS *et al.* Inhaled nitric oxide attenuates acute lung injury via inhibition of nuclear factor-κB and inflammation. *J Appl Physiol* 2002; **92**: 795–801.
88. Meldrum DR, McIntyre Jr RC, Sheridan BC, Cleveland Jr JC, Fullerton DA, Harken HH. L-arginine decreases alveolar macrophage inflammatory monokine production during acute lung injury by a nitric oxide synthase-dependent mechanism. *J Trauma* 1997; **43**: 888–893.