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□ 원 저 □

Mechanism of Decrease in Lung Injury by Low Dose of Endotoxin During Hyperoxia in the Rats

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=국문초록=

저용량의 내독소가 쥐에서 고농도의 산소에 의한 급성폐손상을 경감시키는 기전

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송정섭, 윤형규, 김영균, 김관형, 문화식, 박성학

배 경 : 쥐를 고농도의 산소에 60시간 이상 노출시켰을 때 급성 폐손상이 유발되지만 내독소를 저용량으로 투여시에는 이러한 폐손상이 경감된다고 알려져 있으나 그 기전에 대하여는 확실히 밝혀지지 않고 있다. 산화질소(nitric oxide, NO)는 내독소나 염증성 사이토카인(cytokine) 등의 자극에 의해서 폐내 여러 염증세포에서 만들어지며 이 산화질소는 경우에 따라 우리 몸에 이롭거나 해로운 양면성을 지니고 있다. 저자들은 쥐에서 고농도의 산소에 의한 폐손상이 저농도의 내독소 투여로 경감되는 기전에, 산화질소가 중요한 역할을 하는지 또는 항산화효소나 다른 항염증성 사이토카인이 중요한 역할을 하는지를 규명하고자 하였다.

방 법 : 총 120마리의 쥐(Sprague-Dawley rat)를 24마리씩 5군으로 나누어 대조군은 실내 공기를, 고농도 산소군은 100%의 산소를 60시간 투여하였고 내독소군은 100% 산소 투여시 2일간 저용량의 내독소를 투여하였다. 다른 두 군은 산화질소 합성 억제물인 aminoguanidine(AG)과 N-nitro-L-arginine methyl ester (L-NAME)를 각각 2일간 고농도 산소와 내독소에 더하여 투여하였다. 각각의 군에서 폐손상의 정도와 사망률을 관찰하고 superoxide dismutase(SOD), catalase, nitric oxide, IL-6, IL-11 을 기관지폐포세척액에서 측정하고, 고농도산소 투여군과 내독소 투여군의 폐조직에서 iNOS synthase mRNA의 발현을 비교하였다.

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결 과 :

1. 100%의 산소에 60시간 노출시켰을 때 쥐의 사망률은 8.3% 이었고 내독소 투여군은 4.2%, NAME 투여군이 37.5%, AG 투여군이 25%로 산화질소 합성 억제제에 의하여 사망률의 증가가 관찰되었다.
2. 폐의 손상 정도를 나타내는 폐의 wet/dry 중량비와 늑막액도 100%의 산소에 노출된 군에서 증가되었고 내독소 투여에 의하여 감소되었으며 NAME나 AG 투여군에서는 오히려 증가되었다.
3. 이러한 내독소에 의한 폐손상 억제효과가 항산화효소인 SOD나 catalase, 또는 protective cytokine인 IL-6나 IL-11등의 증가와 관련이 있는지를 관찰하였으나 이들 모두에서 유의한 변화를 관찰하지 못하였다.
4. 산화질소는 100% 산소에 노출시킨 군에서도 증가하였으나 내독소 투여군에서 유의하게 더욱 증가하였고 이는 L-NAME 나 aminoguanidine의 투여시 감소하였다.
5. iNOS mRNA의 발현도 내독소 투여군에서 유의하게 증가하였다.

결 론 : 쥐의 고농소 산소 투여에 의한 폐손상은 저용량의 내독소 투여로 경감되며, 이는 주로 내독소 투여에 의한 iNOS mRNA의 발현을 유도하여 생성된 산화질소의 증가에 기인하는 것으로 생각된다. **(Tuberculosis and Respiratory Diseases 2002, 53:148-160)**

중요 단어 : 고농도 산소, 폐손상, 내독소, 산화질소.

Introduction

Acute lung injury due to hyperoxia is a well-known phenomenon and enhanced production of oxidants from the cells are linked to this lung damage[1]. Administration of bacterial endotoxin to the rats which were exposed to hyperoxia results in increased lung superoxide dismutase activity, decreased hyperoxic lung damage and improvement in survival rate[2]. But the role of nitric oxide (NO) in the protection of lung injury during hyperoxia by endotoxin was not well studied until now. NO is reported to participate in the physiology or pathophysiology of every organ systems. Nitric oxide synthase (NOS) is a key enzyme in the formation of NO and both the constitutive (cNOS) and inducible (iNOS) isoforms have been described in human alveolar and bronchial epithelial cells[3]. The production of iNOS is stimulated by certain microbes[4,5], endotoxin (lipopolysaccharide, LPS)[6], and type 1 cytokines, including IFN-γ and TNF-α[7]. Activation of NF-kB/Rel is critical in the induction of iNOS by endotoxin[8]. Nitric oxide is an important physiological regulator controlling many functions within the pulmonary system[9]. However, overproduction of nitric oxide and nitric-oxide derived oxidants such as peroxynitrite has been associated with pulmonary cellular injury[10-14]. On the other hand, administration of NO has been shown to be beneficial in adult respiratory distress syndrome (ARDS) and experimental models of pulmonary injury. NO also has been shown to reduce microvascular and mucosal injury in experimental models of ischemia-reperfusion injury[15,16] and attenuate lung endothelial injury caused by hyperoxia[17]. These beneficial mechanism can be partially explained by the phenomenon that NO inhibits inflammatory cytokine production by

human alveolar macrophages[18] or inhibits neutrophil accumulation by inhibiting the expression of adhesion molecule[19,20].

Analogs of L-arginine, such as N-nitro-L-arginine tethyl ester (L-NAME) and N-monome-thyl-L-arginine(L-NMMA) inhibits NO synthase (NOS) and has been used in humans as a potential treatment for septic shock[21,22]. These inhibitors are nonselective, resulting in similar inhibition of constitutive NOS (cNOS) and inducible NOS (iNOS). In contrast, aminoguanidine (AG) has a greater inhibitory effect on iNOS than cNOS, with a selectivity of 10- to 100 fold[23,24].

In the present study, we suspect the NO as a possible candidate in the protection of acute lung injury by low dose of endotoxin treatment during exposure to the hyperoxia in the rats. To support the hypothesis, we used the L-NAME and AG as a inhibitor of NOS and observed the relationship between NO inhibition and lung injury, survival rates in the hyperoxic lung injury models.

Materials And Methods

Animals and exposure to hyperoxia

Sprague-Dawley adult male rats weighing 250 to 300 g (n=120) were exposed to continuous flow (7 liters/min) of 100% oxygen for up to 60 hours in 3.5ft^3 clear-plastic exposure chambers. Control animals were raised in room air under normal vivarium conditions for 60 hours.

Endotoxin and nitric oxide synthase inhibitor treatment

Endotoxin (E.coli lipopolysaccharide 055:B5, 500 ug/Kg), L-NAME (30 mg/Kg), or aminoguanidine (300 mg/Kg) was given by intraperitoneal injection for 2 times at 0 and 24 hours after the beginning of hyperoxic exposure.

Lung analyses

All animals were anesthetized with pentobarbital sodium, 70 mg/Kg. The lungs were perfused with 0.9% NaCl through the pulmonary artery after the abdominal aorta was transected. After measuring the volume of pleural fluid, broncho-alveolar lavage was done with 10 ml of cold HBSS (Hanks Balanced Salt Solution) for 5 times. Then Rt lung was weighed and wet/dry weight ratio was calculated after measuring the dry weight 48 hours after placing the lung in a vacuum oven at 60℃. Lt lung was immediately put in the liquid nitrogen and later kept at the -70℃. The lung was homogenized for 2 min. in a cold homogenizer with a solution of potassium phosphate buffer, 0.005 M pH 7.8 for the assay of SOD, catalase and Northern blot assay for iNOS mRNA. BAL fluid was centrifuged and the supernatants were also kept at the -70℃ for the later assay of IL-6, IL-11 and nitric oxide.

SOD assay

One unit of SOD is defined as that which causes 50% inhibition of the xanthine-xanthine oxidase mediated reduction of ferricytochrome C. The assay is performed in 3 ml of 20 mM sodium carbonate buffer at pH 10, 0.1 mM

EDTA, 1 uM of ferricytochrome C, 50 uM of xanthine, and sufficient xanthine oxidase to produce a rate of reduction of ferricytochrome C at 550 nm of 0.025 absorbance unit per min[25].

Catalase assay

Catalase activity was measured by measuring the decomposition of H_2O_2 at wavelength 240 nm[26]. The reaction mixture was consisted of 30 mM H_2O_2, 0.05 M phosphate buffer, pH 7.0. Units of catalase activity was calculated by comparing the standard curve which was made directly from the known concentration of catalase standard (bovine liver, Sigma Co.).

Nitric oxide assay

Nitrite production was assayed after the incubation period by measuring the accumulation of nitrite in the BAL fluid using the Griess reaction. Briefly, an aliquot (100 ul) of the BAL fluid was mixed with an equal volume of Griess reagent (sulfanilamide, 1% wt/vol; naphthylethylenediamine dihydrochloride, 0.1% wt/vol; and orthophosphoric acid, 2.5% vol/vol) and was incubated at room temperature at 10 min. The absorbance was read at 540 nm at ELISA reader. In order to reduce the nitrate to nitrite, aliquot (50 ul) of BAL fluid was mixed with equal amount of nitrate reductase buffer (0.1 U/ml nitrate reductase, 50 uM NADPH, 5 uM FAD), and the reaction was continued for a 2 hour at room temperature. The newly converted nitrite was determined as above by addition of 100 ul of Griess reagent. Nitrite was determined using sodium nitrite as a standard.

Cytokine determinants

Concentrations of IL-6 and IL-11 in BAL fluids were measured by a ELISA kits (R&D, USA).

Northern blotting

The lung homogenate in Ultraspec-II Reagnet (Biotecx, Houston, Texas) was centrifuged at 12,000g. The yield and concentration of RNA were determined by spectroscopic measurement of absorbance at 260 nm. Twenty microgram of RNA per rat was loaded in the gel. The RNA was analyzed by standard Northern blot and hybridization techniques with the DIG-labeled iNOS cDNA probe. The cDNA probe was synthesized from the RNA of lipopolysaccharide and interferon treated RAW264.7 macrophages and then DIG labeled using the PCR DIG probe synthesis kit(Roche, Germany). The cDNA probe was denatured by boiling and then hybridized to the blots overnight at 65℃. After the blots were washed at high stringency, a hybridized probe was detected.

Statistical analysis

All results are expressed as mean±SEM. Comparisons of various parameters between groups were tested for their significance with the three-way analysis of variance (ANOVA). Differences were regarded as significant if $p<0.05$.

Table 1. Mortality of the rats in the five groups

	Survived	Death	Mortality
Room air (n=24)	24	0	0%
Hyperoxia (n=24)	22	2	8.3%*
O_2+LPS (n=24)	23	1	4.2%
O_2+LPS+NAME (n=24)	15	9	37.5%*
O_2+LPS+AG (n=24)	18	6	25%*

* Signicantly different from air control groups, $p < 0.01$.

Table 2. Comparative lung changes in adult rats exposed to 100 % O_2 for 60 hours.

Group	Pleural Effusion, ml	Lung Wet/Dry Wt
Room air control	0	9.82 ± 0.91
O_2	5.38 ± 3.27*	12.69 ± 1.64*
O_2 + LPS	0.54 ± 1.24	10.73 ± 1.10
O_2 + LPS + NAME	1.29 ± 1.56	12.69 ± 1.56*
O_2 + LPS + AG	1.46 ± 1.56	13.09 ± 3.78*

Values are means±SD. Pleural effusion was determined by absorbing fluid from both chest cavity with the syringe with 18 gauge catheter. Lung wet/dry weight ratio was calculated by measuring the dry weight after 48 hours in oven at 60°C.

* Significantly different from room air control groups. $P < 0.05$.

Results

Effects of endotoxin with or without nitric oxide synthase inhibitor in the hyperoxia-exposed rats.

As expected, low dose endotoxin decreased the mortality from the hyperoxia exposed rats but L-NAME or aminoguanidine markedly increased the mortality rate. Data on mortality according to the different groups are summarized in (**Table 1**). Among the alive rats, lung injury was markedly increased by exposure to hyperoxia for 60 hours; the lung wet/dry weight ratio of control was 9.8 ±0.9, and this ratio was markedly increased to 12.7±1.6 by exposure to hyperoxia. Lipopolysaccharide (LPS) decreased the ratio to 10.7± 1.1. In the case of adding the N-nitro-L-arginine methyl ester (L-NAME) or aminoguanidine (AG), the wet/dry ratio was increased to 12.7±1.6 and 13.1±3.8 (**Table 2**).

Pleural fluids were also markedly increased in the hyperoxia exposed groups (5.383.27 ml) than in control groups (0 ml) and lipopolysaccharide decreased the pleural effusion (**Table 2**).

Superoxide dismutase(SOD) and catalase activity

SOD and catalase activity were expressed as unit/mg protein. The protein amounts in the homogenized lung tissue was measured by Bio-Rad protein assay kit(Bio-Rad, USA). The

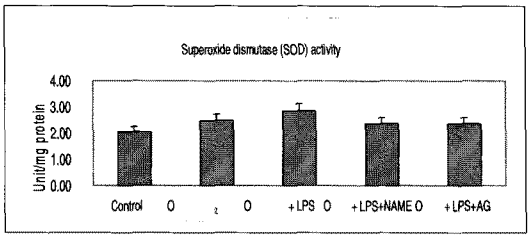

Fig. 1. Lung SOD activity in the control and hyperoxia exposed rats with or without treatments. Results were expressed as SOD activity per mg protein. The SOD activity from each treated groups were not different from the room air control group.

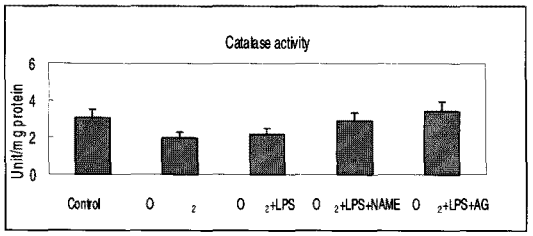

Fig. 2. Lung catalase activity of hyperoxia exposed rats with or without treatments. Results are expressed as units/mg protein. There were no significant differences between different groups.

lung SOD activity **(Fig. 1)** and catalase activity **(Fig. 2)** from each treated groups were not different from the control group.

IL-6 and IL-11

IL-6 and IL-11 is known as protective cytokine against acute lung injury. IL-6 level was slightly increased in hyperoxia plus LPS group (106.4 ± 89.5 pg/ml) than control group (38.9±35.0 pg/ml) or hyperoxia alone group (42.1±8.0 pg/ml) without significance ($p>0.05$) **(Fig. 3).** There were no specific differences in the IL-11 levels between different groups ($p>0.05$) **(Fig. 4).**

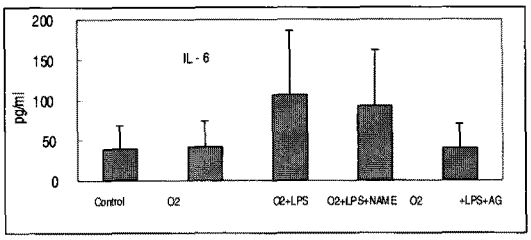

Fig. 3. Effects of different treatments on the IL-6 levels of bronchoalveolar lavage fluid in the rats. IL-6 levels are expressed as pg/ml. The IL-6 levels were slightly increased in the hyperoxia and lipopolysaccharide treated groups than room air control group ($p>0.05$).

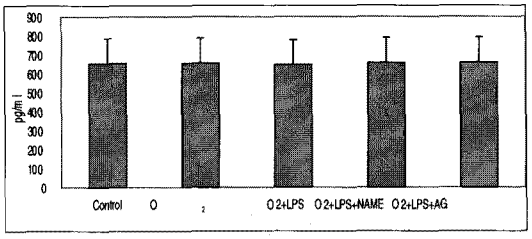

Fig. 4. Effects of different treatments on the IL-11 levels of bronchoalveolar lavage fluid in the rats. IL-11 levels are expressed as pg/ml. There were no specific differences between groups.

Measurements of nitric oxide (NO) production

Hyperoxia and hyperoxia plus lipopolysaccharide increased the NO production in the bronchoalveolar lavage fluid of the rats. The treatment of nitric oxide synthase inhibitor, L-NAME or aminoguanidine significantly decreased the NO production **(Fig. 5).**

Effects on iNOS mRNA

Northern blot analysis was used to determine the iNOS mRNA expression from the lung

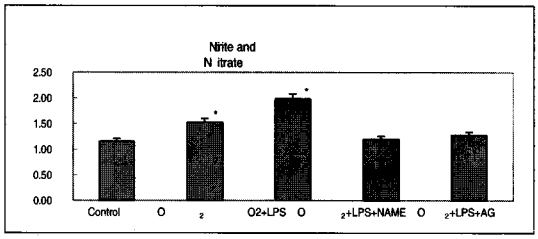

Fig. 5. NO production by hyperoxia or hyperoxia plus LPS with or without L-NAME or aminoguanidine. The nitrite and nitrate concentrations in the bronchoalveolar lavage fluids were measured as described in METHODS. NO production was increased in hyperoxia group (1.53±0.45 uM) and hyperoxia plus LPS group (1.99±0.59 uM) than from control group (1.15±0.25 uM). The increased NO production by LPS was markedly decreased by nitric oxide synthase inhibitor, L-NAME or aminoguanidine to 1.20±0.41 uM and 1.28±0.50 uM respectively. * Statistically significant increase in nitrite and nitrate production compared with room air control ($p<0.05$).

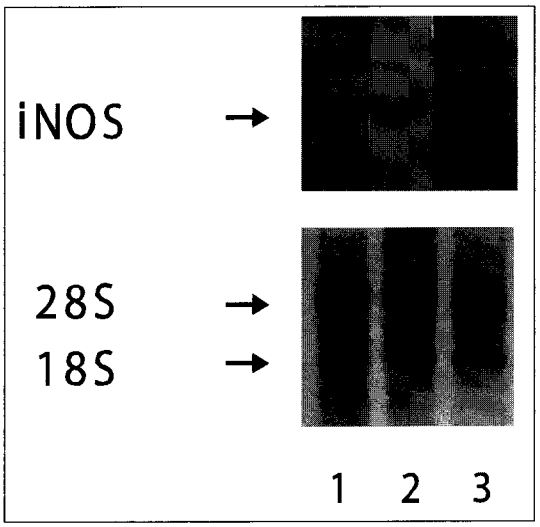

Fig. 6. Northern blot of iNOS mRNA in rat lung. RNA was extracted from right lung using Ulraspec-II (Biotecx, Houston, Texas, USA). Twenty micrograms of total RNA was electrophoresis on 1% agarose containing formaldehyde and were transferred to nylon membrane by blotting. The membrane was hybridized with a DIG-labeled probe specific for iNOS cDNA.
Lane 1: Control, Lane 2: hyperoxia,
Lane 3: Hyperoxia + LPS.

tissues. A representative Northern blot is shown in **Fig. 6**. This result demonstrates that there was no iNOS message in rat lungs exposed to room air **(Fig. 6, lane 1)**. Exposure of the rats to hyperoxia for 60 hours or hyperoxia plus LPS led to a substantial level of iNOS mRNA **(Fig. 6, lane 2 and lane 3)**.

Discussion

The findings in this study suggest that low dose endotoxin protect the lung from the hyperoxia exposed rats by iNOS mRNA induction and NO production. Neither the known protective cytokine IL-6, IL-11 nor the antioxidant superoxide dismutase(SOD), catalase were not increased by low dose endotoxin in hyperoxia exposed rats.

Administration of bacterial endotoxin in the hyperoxia exposed rats prevents lung damage and increase the survival rate[2]. This protective mechanism is still unknown although there was a paper that SOD is involved in this protection[2].

Nitric oxide(NO) is generated in large amounts by inducible nitric oxide synthase(iNOS) which is usually only produced after stimulation of alveolar macrophages by tumor necrosis factor-α (TNF-α), IL-1β, interferon-γ or lipopolysaccharide(LPS). The synthesis of small amounts of NO in the vascular endothelium, catalyzed by

a calcium dependent isoform (eNOS), is regarded as a key regulator of vascular tone and integrity, and of regional blood flow[34]. The iNOS inhibition may prevent endothelial dysfunction, so NO may be cytotoxic in certain circumstances[28]. By contrast, NO may protect against lung injury via the inhibition of neutrophil adhesion to the endothelium, and through its scavenging capacity for reactive oxygen species[29,30]. In this experiment, low dose LPS decreased mortality and lung injury from the hyperoxia exposed rats and NOS inhibitor, L-NAME or aminoguanidine (AG) markedly increased the mortality **(Table 1)** and lung injury (Fig. 1). These findings are different from the previous paper that L-NAME or aminoguanidine significantly attenuate the NNMU (N-nitroso-N- methylurethane) induced alveolar injury in rats[31] and ozone induced airway inflammation in guinea pigs[32] and mice[33]. Aminoguanidine, containing the guanido-group of L-arginine linked to hydrazine, in vitro, displays 10 to 100 fold higher potency as inhibitor of iNOS than of eNOS[35]. In endotoxemic rodents and dogs, AG suppresses activation of iNOS and peroxynitrite production in the lungs and decreases plasma levels of nitrite and nitrates. Moreover, AG reduces lung edema, improves gas exchange, and increased survival by counteracting circulatory failure[36-38]. In contrast, depletion of the 5' region of the iNOS gene in mice is associated with a high leukocyte count and exacerbates hyperoxia-induced lung injury[39]. Reduced lung injury was associated with increased levels of iNOS mRNA expression in hyperoxia exposed mice[39]. LPS, in association with inflammatory cytokines such as IL-1β, TNF-α or interferon-γ induced nitric oxide synthase[18].

The current study revealed that hyperoxia alone induced iNOS mRNA but two times of intraperitoneal injection with low dose LPS(500 ug/Kg) markedly increased the iNOS mRNA expression and NO production. This increased production of NO by low dose of endotoxin might be due to interaction with IL-1 or TNF which were released from the activated alveolar macrophages in the hyperoxic environment or by endotoxin stimulation.

Recently, NO inhalation has been used to improve arterial blood oxygenation in patients with adult respiratory distress syndrome. High levels of IL-8 and IL-6 were decreased in these ARDS patient's bronchoalveloar lavage fluid after NO inhalation[47]. In addition, endogenous NO limits cytokine-induced damage of murine lung epithelial cells[48]. These cytoprotective effects of NO, such as inhibition of neutrophil or platelet adhesion to the endothelium are usually attributed to the constitutive NOS activity. In contrast, higher concentrations of NO, produced by activation of iNOS during the inflammatory process, are generally thought to be cytotoxic. But, the results from our study suggest that NO produced by iNOS during endotoxin treatment in hyperoxia exposed rats decrease the lung injury and increase the survival ; therefore the induced form of NOS might serve as a protective role such as endogenous NOS. NO inhibit the neutrophil oxidants production via a direct effect on NADPH oxidase[49] and by reducing the availability of ferrous iron[50]. NO also increase the cellular glutathione levels in rat lung fibroblasts[51] and may contribute to a protective

effect against oxidant injury. Our data also show that aminoguanidine, a selective iNOS inhibitor and L-NAME, nonselective cNOS inhibitor markedly increased the lung injury and mortality.

The etiology of lung injury during prolonged exposure to high doses of oxygen appears to be mediated by free radicals because antioxidant enzymes which was induced by administration of endotoxin improves survival of animals exposed to hyperoxia[40, 41]. In addition, Inflammatory cytokines such as tumor necrosis factor (TNF) and intercellular adhesion molecule-1 (ICAM-1) is known to be involved in hyperoxic lung injury[42, 43]. We measured lung tissue homogenate antioxidant enzyme, superoxide dismutase and catalase activities and there were no increases in the antioxidant activities among the endotoxin-treated and hyperoxia exposed rats. This differences might be due to species variations because rat strain differences affect the lung to develop edema after exposure to hyperoxia[44, 45]. Among the cytokines, IL-6 induced protection in hyperoxic acute lung injury in mice[27] and IL-11 also protect the lung in the 100% oxygen exposure[46]. The mechanism of protection in hyperoxic acute lung injury by IL-6 is either increase the Mn SOD activities[52] or associated with induction of cell-death inhibitor, Bcl-2 and tissue inhibitor of metalloproteinase-1 (TIMP-1)[27]. IL-11 also attenuates lung inflammation by preventing neutrophil sequestration, TNF-α production and pulmonary vasomotor dysfunction in endotoxin-induced lung injury[53].

In our experiment, these protective cytokines IL-6, IL-11 and antioxidant enzymes including SOD and catalase were not increased by endotoxin treatment during hyperoxic exposure in the rats.

In summary, our studies show that low dose endotoxin treatment has impressive protective effects in the setting of 100% oxygen induced acute lung injury in the rats. These protective effects were mediated by iNOS induction and nitric oxide production and not by protective cytokines, SOD and catalase. These results suggest that exogenously administered nitric oxide or iNOS mRNA induction by cytokines might be useful to protect the lung in the setting of hyperoxia exposed states.

References

1. Freeman BA, Crapo JD. Biology of disease: free radicals and tissue injury. Lab Invest 1982; 47:412-26.
2. Hass MA, Frank L, Massaro D. The effect of bacterial endotoxin on synthesis of (Cu,Zn)superoxide dismutase in lungs of oxygen-exposed rats. J Biol Chem 1981;257: 9379-83.
3. Asano K, Chee CBE, Gaston B, Lilly CM, Gerald C, Drazen JM, Stamler J. Constitutive and inducible nitric oxide synthase gene expression, regulation and activity in human lung epithelial cells. Proc Natl Acad Sci USA 1994;91:10089-93.
4. Liew F, Millott S, Parkinson C, Palmer R, Moncada S. Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J immunol 1990;144: 4794-7

5. Lovchik J, Lyons C, Lipscomb M. A role for gamma interferon-induced nitric oxide pulmonary clearance of cryptococcus neoformans. Am J Respir Cell Mol Biol 1995;13:116-24
6. Liu S, Adcock I, Old R, Barns P, Evans T. Lipopolysaccharide treatment in vivo induces widespread tissue expression of inducible nitric oxide synthase mRNA. Biochem Biophys Res Commun 1993;196:1209-13
7. Nathan C, Xie O. 1994. Regulation of biosynthesis of nitric oxide. J Biol Chem 1994; 269:13725-28
8. Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-kB/Rel in induction of nitric oxide synthase. J Biol Chem 1993;269:4705-8
9. Gaston B, Drazen JM, Loscalzo J, Stanler JS. The biology of nitrogen oxides in the airways. Am J Respir Crit Care Med 1994; 149:538-51.
10. Bernareggi M, Mitchell JA, Barnes PJ, Belvici MG. Dual action of nitric oxide on airway plasma leakage. Am J Respir Crit Care Med 1997;155:869-74.
11. Garat C, Jayr C, Eddahibi S, Laffon M, Meigan M, Adnot S. Effect of inhaled nitric oxide or inhibition of endogenous nitric oxide formation on hyperoxic lung injury. Am J Respir Crit Care Med 1997;155:1957-64.
12. Haddad IY, Pataki G, Hu P, Galliani C, Beckman JS, Matalon S. Quantitation of nitrotyrosine levels in lung sections of patients and animals with acute lung injury. J Clin Invest 1994; 94:2407-13.
13. Ischiropoulos H, Al-Mehdi AB, Fisher AB. Reactive species in rat lung injury: contribution of peroxynitrite. Am J Physiol 1995;269(Lung Cell Mol Physiol 13):L155-L64.
14. Gow AJ, Thom SR, Ischiropoulos H. Nitric oxide and peroxynitrite-mediated pulmonary cell death. Am J Physiol 1998;274 (Lung Cell Mol Physiol 18):L112-L8.
15. Kurose I, Kubes P, Wolf R, Anderson D, Paulson J, Miyazaka M, Granger D. Inhibition of nitric oxide production: mechanisms of vascular albumin leakage. Cric Res 1993;73:164-71.
16. Kurose I, Wolf R, Grisham M, Granger D. Modulation of ischemia/reperfusion-induced micro-vascular dysfunction by nitric oxide. Circ Res 1994;74:376-82.
17. McElroy MC, WienerKronish JP, Miyazaki H, Sawa T, Modelska K, Dobbs LG, Pittet JF. Nitric oxide attenuates lung endothelial injury caused by sublethal hyperoxia in rats. Am J Physiol 1997;172 (Lung Cell Mol Physiol 16):LL631-L8.
18. Thomassen MJ, Buhrow LT, Connors MJ, Kaneko T, Erzurum SC, Kavuru MS. Nitric oxide inhibits inflammatory cytokine production by human alveolar macrophages. Am J Respir Cell Mol Biol 1997;17:279-83.
19. Caterina D, Libby RP, Peng H, Thannickal VJ, Rajavashisth TB, Gimbrone MA, Shin WS, Liao JK. Nitric oxide decreases cytokine-induced endothelial activation. J Clin Invest 1995; 96:60-8.
20. Guidot DM, Hybertson BM, Kirlowski RP, Repine JE. Inhaled NO prevents IL-1-induced neutrophil accumulation and associated acute edema in isolated rat lungs. Am J Physiol 1996; 271 (Lung Cell Mol Physiol

15):L225-L9.

21. Schilling J, Cakmakci M, Battig U, Geroulanos G. A new approach to the treatment of hypotension in septic shock by NG-monomethyl-L-arginine, an inhibitor of nitric oxide synthase. Intensive Care Med 1993;19:227-31.
22. Peters A, Lamb G, Leone A, Moncada S, Bennett D, Valance P. Effect of a nitric oxide synthase inhibitor in humans with septic shock. Cardiovasc Res. 1994;28:34-9.
23. Misko TP, Moore WM, Kasten TP, Nichols GA, Corbett JA, Tilton RG, McDaniel ML, Williamson JR, Currie MG. Selective inhibition of inducible nitric oxide synthase by aminoguanidine. Eur J pharmacol 1993; 233:119-25.
24. Hasa Nk, Heesen BJ, Corbett JA, McDaniel ML, Chang K, Allison W, Wolffenbuttel BH, Williamson JR, Tilton BG. Inhibition of nitric oxide formation by guanidines. Eur J Pharmacol 1993;249:101-6.
25. McCord JM, Fridovich I. Superoxide dismutase. An enzymatic function for erythropocuprein (hemocuprein). J Biol Chem 1969;25: 6049-55.
26. Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121-6.
27. Eard NS, Waxman AB, Homer RJ, Mantell LL, Einarsson O, Du Y, Elias JA. Interleukin-6 induced protection in hyperoxic acute lung injury. Am J Respir Cell Mol Biol 2000;22:535-42.
28. Worral NK, Chang K, Suau GM, Allison WS, Pmisko T, Sullivan PM, Tilton RG, Williamson JR, Feruson,Jr TB. 1996. Inhibition of inducible nitric oxide synthase prevents myocardial and systemic vascular barrier dysfunction during early cardiac allograft rejection. Cir Res 1996; 78:769-79.
29. Okabayashi K, Triantafillou AN, Yamashita M, Aoe M, DeMeester SR, Cooper JD, Patterson GA. Inhaled nitric oxide improves lung allograft function after prolonged storage. J Thorac Cardiovas Surg. 1996;112: 293-9.
30. Kurose I, Wolf R, Grisham MB, Granger DN. Modulation of ischemia/reperfusion-induced microvascular dysfunction by nitric oxide. Circ Res 1994;74:376-82.
31. Cruz WS, Moxley MA, Corbett JA, Longmore WJ. Inhibition of nitric oxide synthase attenuates NNMU-induced alveolar injury in vivo. Am J Physiol 1997;273: -L1167-73.
32. Inoue H, Aizawa H, Nakano H, Matsumoto K, Kuwano K, Nadel JA., Hara N. Nitric oxide synthase inhibitors attenuate ozone-induced airway inflammation in guinea pigs: possible role of interleukin 8. Am J Respir Crit Care Med 2000;161:249-56.
33. Fakhrzaeh L, Jeffrey D, Laskin, 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;26:413-19.
34. Moncada S, Palmer RM, Higgs EA. Nitric oxide:physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43:109-42.
35. Misko TP, Moore WM, Kasten TP, Nickols GA, Corbett JA, Tilton RG, McDaniel ML, Williamson JR, Currie MG. Eur J Pharmacol 1993;233:119-25.

36. Numata M, Suzuki S, Miyazawa N, Miyashita A, Nagashima Y, Inoue S, Kaneda T, Okubo T. Inhibition of inducible nitric oxide synthase prevents LPS-induced acute lung injury in dogs. J Immunol 1998;160: 3031-7.
37. 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: 905-11.
38. Evgenov OV, hevroy O, Bremnes KE, Bjertnes LJ. Effect of aminoguanidine on lung fluid filtration after endotoxin in awake sheep. Am J Respir Crit Care Med 2000; 162:465-470.
39. Kobayashi H, Hataishi R, Mitsufuji H, Tanaka M, Jacobson M, Tomita T, Zapol WM, Jones RC. Antiinflammatory properties of inducible nitric oxide synthase in acute hyperoxic lung injury. Am J Respir Cell Mol Biol 2001;24:390-7.
40. Frank L, Summerville J, Masaro D. Protection from oxygen toxicity with endotoxin. Role of endogenous antioxidant enzymes of the lung. J Clin Invest 1980;65:1104-10.
41. Frank L, Yam J, Roberts RJ. The role of endotoxin in protection of adult rats from oxygen-induced lung toxicity. J Clin Invest 1978;61:269-75.
42. Jensen JC, Pogrebniak HW, Pass HI, Buresh C, Merino MJ, Kauffman D, Venzon D, Langstein HN, Norton JA. Role of tumor necrosis factor in oxygen toxicity. J Appl Physiol 1992;72:1902-7.
43. Welty SE, Rivera JL, Elliston JF, Smith CV, Zeb T, Ballantyne CM, Montgomery CA, Hansen N. Increases in lung tissue expression of intercellular adhesion molecule-1 are associated with hyperoxic lung injury and inflammation in mice. Am J Respir Cell Mol Biol 1993;9:393-400.
44. Bryan CL, Jenkinson SG. Species variation in lung oxidant enzyme activities. J Appl Physiol 1987; 63:597-602.
45. Lishan HE, Chang SW, Ortiz P, Montellano DE, Burke TJ, Voelkel NF. Lung injury in Fisher but not Sprague-Dawley rats after short-term hyperoxia. Am J Physiol 1990;259: L451-L8.
46. Waxman AB, Einarsson O, Seres T, Knickelbein RC, Warshaw JB, Johnson R, Homer RJ, Elias JA. Targeted lung expression of interleukin-11 enhances murine tolerance of 100% oxygen and diminishes hyperoxia-induced DNA fragmentation. J Clin Invest 1998;101:1970-82.
47. Chollet-Martin S, Gatecel C, Kermarrec N, Gougerot-Pocidalo MA, Didier MP. Alveolar neutrophil functions and cytokine levels in patients with the adult respiratory distress syndrome and during nitric oxide inhalation. Am J Respir Crit Care Med 1996;153:985-90.
48. Burke-Gaffney A, Hellewell PG. Endogenous nitric oxide limits cytokine-induced damage of murine lung epithelial cells. Am J physiol 1997;272:L707-L13.
49. 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:

1116-21.

50. Kanner J, Harrel S, Granit R. Nitric oxide as an antioxidant. Arch Biochem Biophys 1991; 289:130-6.
51. White AC, Maloney EK, Boustani MR, Hassoun PM, Fanburg BL. Nitric oxide increases cellular glutathione levels in rat lung fibroblasts. Am J Respir Cell Mol Biol 1995;13:442-8.
52. Tsan MF, Ehite JE, Del Vecchio PJ, Shaffer JB. IL-6 enhances TNF-αand IL-1 induced increase of Mn superoxide dismutase mRNA and O_2 tolerance. Am J Physiol 1992;263: L22-L6.
53. Sheridan BC, Dinarello CA, Meldrum DE, Fullerton DA, Selzman CH, McIntyre JR RC. Interleukin-11 attenuates pulmonary inflammation and vasomotor dysfunction in endotoxin-induced lung injury. Am J Physiol 1999;277:L861-L7.