Nitric oxide and hyperoxic acute lung injury

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Abstract

Hyperoxic acute lung injury (HALI) refers to the damage to the lungs secondary to exposure to elevated oxygen partial pressure. HALI has been a concern in clinical practice with the development of deep diving and the use of normobaric as well as hyperbaric oxygen in clinical practice. Although the pathogenesis of HALI has been extensively studied, the findings are still controversial. Nitric oxide (NO) is an intercellular messenger and has been considered as a signaling molecule involved in many physiological and pathological processes. Although the role of NO in the occurrence and development of pulmonary diseases including HALI has been extensively studied, the findings on the role of NO in HALI are conflicting. Moreover, inhalation of NO has been approved as a therapeutic strategy for several diseases. In this paper, we briefly summarize the role of NO in the pathogenesis of HALI and the therapeutic potential of inhaled NO in HALI.

Key words: hyperoxic acute lung injury; hyperoxia; nitric oxide; nitric oxide synthase; reactive oxygen species; reactive nitrogen species; inhaled nitric oxide; nitro-L-arginine methyl ester

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Introduction

It is well known that oxygen is indispensable for human life. In clinical practice, oxygen therapy is also one of the main supportive modalities for the diseases with presentations of hypoxemia. However, oxygen also has affinity for electrons and may cause damage to cells and tissues, especially in the presence of endogenous organometallic coordination compounds that can catalyze the incomplete reduction of oxygen (Allen et al., 2009). If the body is exposed to oxygen partial pressure (\(P_{O_2}\)) at a level higher than normal atmosphere for enough time, the defenses may be compromised, and oxygen toxicity will occur (Allen et al., 2009; Thomson and Paton, 2014). This concern increases with the development of deep diving and the use of normobaric [\(P_{O_2} = 1\) atmosphere absolute (ATA); 1 ATA = 100 kPa] as well as hyperbaric oxygen (\(P_{O_2} > 1\) ATA) in clinical practice (Hu et al., 2015; Stoller, 2015). Usually, oxygen at elevated partial pressure may cause damage to the central nervous system (CNS) (Hampson and Atik, 2003), respiratory system (especially the lung) (Kallet and Matthay, 2013) and eyes (Nichols and Lambertsen, 1969). Of them, the hyperoxic acute lung injury (HALI) is more common in clinical practice.

Currently, available studies (Lv et al., 2014; Weaver and Liu, 2015) have indicated the pathogenesis of HALI is complex, involving reactive oxygen species (ROS)/reactive nitrogen species (RNS), inflammation, cell apoptosis, autophagy. Nitric oxide (NO) is an important component of RNS and plays an important role in the pathogenesis of HALI. Although the roles of NO and its synthetases in the pathogenesis of HALI have been investigated extensively, the available findings are still controversial. In addition,
exogenous NO (inhaled NO, iNO) has been also used in the treatment of HALI in premature infants (Ballard et al., 2006b). Herein, we briefly summarize the role of NO in HALI.

**Introduction of NO**

NO is also known as the endothelium-derived relaxing factor and has been found as an omnipresent intercellular messenger in all vertebrates and a signaling molecule that is involved in a variety of physiological and pathological processes (Fu et al., 2014). The biological production of NO in the body is also crucial for the non-specific host defense (Culotta and Koshland, 1992; Pacher et al., 2007).

Endogenously NO is biosynthesized from L-arginine, oxygen, and NADPH in the catalysis of nitric oxide synthases (NOS) (Pacher et al., 2007; Bogdan, 2015). To date, three isoforms of NOS have been identified. Neuronal NOS (nNOS) is formed constitutively in the central and peripheral nervous systems (Li et al., 2014), and its activity is calcium dependent. The other constitutive isoform of NOS, endothelial NOS (eNOS), is also calcium dependent and is expressed constitutively by a variety of cells, including the pulmonary vascular endothelial cells (Pacher et al., 2007). The tonic production of NO by eNOS is important for the maintenance of blood flow in some organs such as the heart, lung, liver and kidneys (Culotta and Koshland, 1992). In addition, NO derived from eNOS is able to regulate the platelet and neutrophil adhesion and activation (Culotta and Koshland, 1992). The third isoform of NOS is inducible NOS (iNOS) that is calcium-independent. iNOS activity is induced by a variety of pro-inflammatory cytokines both in vitro and in vivo, including tumor necrosis factor (TNF), interferon (IFN) gamma and interleukin-1 (IL-1) (Pacher et al., 2007; Liu et al., 2014; Bogdan, 2015). iNOS expression/activity is also induced both in vitro and in vivo by administration of bacterial endotoxin (lipopolysaccharide, LPS) (Bogdan, 2015). However, the regulation of iNOS expression varies with the specific cell type or animal model studied.

All the NOS isoforms are constitutively expressed in human airways. eNOS is constitutively expressed in human bronchial epithelial cells as well as type II human alveolar epithelial cells (Shaul et al., 1994), and abundant eNOS immunoreactivity was found in endothelial cells of pulmonary blood vessels. In situ hybridization and immunohistochemistry show iNOS is expressed in airway epithelial cells of resected lung tissues and specimens obtained bronchoscopically (Kobzik et al., 1993; Guo et al., 1995). Although the activated macrophages can express iNOS, alveolar macrophages appear to have little or no basal iNOS expression (Guo et al., 2000; Lane et al., 2004). Immunohistochemistry for nNOS has localized it to nerves of the inhibitory non-adrenergic noncholinergic system in the airway smooth muscle (Ward et al., 1995) and the submucosa (Kobzik et al., 1993). Immunohistochemically, extraneuronal nNOS is described in the airway epithelium (Ricciardolo et al., 2001) and endothelium (Luhrs et al., 2002).

**Biological activities of NO**

The major signal transduction mechanism of NO has been ascribed to the production of cyclic guanosine monophosphate (cGMP) by guanylate cyclase (Pacher et al., 2007). NO is a small hydrophobic molecule and can cross cell membranes without the help of channels or receptors. NO can diffuse into surrounding cells and then activate soluble guanylate cyclase in the target tissues leading to the production of cGMP. In turn, cGMP activates cGMP dependent kinases in the target tissues, which may modulate the intracellular calcium levels and regulate a variety of diverse activities in the target tissues.

NO is a potent oxidant or a free radical, and many of its potentially toxic effects are more likely mediated by its oxidation products rather than NO itself. NO can react with superoxide anion to form the potent oxidant peroxynitrite (ONOO-). ONOO- production has been demonstrated in the lungs of rodents exposed to prolonged hyperoxia (Haddad et al., 1994).

NO is a critical mediator of vasodilation. The regulation of extracellular fluid homeostasis and the control of blood flow and blood pressure are largely dependent on the vasodilatory actions of NO (Bredt, 1999). The vasodilatory effect of NO is also crucial for the development and maintenance of penile erection, which is the biological basis of sildenafil (Viagra), which acts to inhibit the phosphodiesterase type 5 to lower the cGMP concentration by converting it back to GMP (Burnett, 2008).

NO and other RNS can react with structural elements, components of the replication machinery, nucleic acids, metabolic enzymes, or with virulence-associated molecules of infectious pathogens, which is the basis for their direct anti-viral or anti-microbial effects (Pacher et al., 2007). In addition, NO is crucial for the T and B cell differentiation, and tumor defense (Bogdan, 2015).

NO also serves as a neurotransmitter between nerve cells. As an important nonadrenergic noncholinergic neurotransmitter, NO may also cause the relaxation of the gastrointestinal smooth muscle (Bult et al., 1990).

Under physiological conditions, endogenous NO may tonically suppress the microvascular permeability in the airway mucosa, but it is unlikely that NO is a significant endogenous regulator of basal airway tone. In contrast, NO is crucial in the regulation of airway responsiveness.
(Redington, 2006). In addition, NO is also involved in the lung inflammation, but the available findings on this issue are still controversial, which may be ascribed to the use of the relative selectivity of the different NOS inhibitors (Redington, 2006).

**NO AND LUNG DISEASES**

iNOS has been considered as the principal NOS isoform and a therapeutic target in lung diseases, but increasing evidence suggests its role of the “constitutive” NOS isoforms in airways diseases (Barnes, 1995). Findings have shown that nNOS is involved in the development of allergen-induced hyper-responsiveness and that eNOS may regulate the microvascular permeability in inflammation, but further exploration is warranted (Redington, 2006). Studies have described the relationships between the eNOS (Lee et al., 2000; Yanamandra et al., 2005) and nNOS (Grasemann et al., 1999) gene polymorphisms and the asthma phenotype. On the other hand, nNOS derived NO was found to inhibit the proliferation of human airway smooth muscle cells in vitro (Patel et al., 1999; Hamad and Knox, 2001) and chronic blockade of this activity might therefore deteriorate airway dysfunction via facilitating the hypertrophy and hyperplasia of airway smooth muscle (Redington and Howarth, 1997). Nitrotyrosine is a so-called footprint of nitrosative stress. In the study of Haddad et al. (1994), immunohistochemistry showed nitrotyrosine staining in the lung of acute respiratory distress syndrome (ARDS) patients but not patients with other conditions. Findings from the study of Sittipunt et al. (2001) showed the concentrations of nitrite and nitrate in the bronchoalveolar lavage fluid (BALF) were increased in ARDS patients and those at risk for the development of ARDS. Similar findings were also present in the study of Zhu et al. (2001), concentrations of nitrite and nitrate and nitrated pulmonary surfactant protein in the pulmonary edema fluid were higher in ARDS patients than in those with cardiogenic pulmonary edema. In 23 subjects with asthma, immunoreactive iNOS in airway epithelial cells of bronchoalveolar biopsy specimens was found in 22 patients, but only 2 of 20 healthy non-smoking controls were positive for iNOS (Hamid et al., 1993). In untreated asthma patients, Redington et al. (2001) found both iNOS mRNA and protein expressions were increased. In contrast, the iNOS mRNA and protein expression was comparable between non-asthmatic controls and asthmatic subjects receiving regular maintenance treatment with inhaled corticosteroids. In resected peripheral lung tissues of patients with severe chronic obstructive pulmonary disease (COPD), alveolar macrophages, airway smooth muscle cells, and cells in the alveolar walls identified as type 2 pneumocytes were found to have iNOS expression in immunohistochemistry (Maestrelli et al., 2003) and more iNOS-positive type 2 pneumocytes were found in a control group of smokers without airflow obstruction. eNOS expression was also reported in alveolar macrophages from COPD patients (van Straaten et al., 1998). In rats with LPS induced lung injury, Arkovitz et al. (1996) found delayed treatment with two different isoform-selective iNOS inhibitors was able to alleviate acute lung injury.

**NO AND HALI**

The role of NO in the pathogenesis of HALI has been extensive studied in animal studies and cell experiments, but the findings are still controversial. In these studies, most focus on the role of iNOS and thus therapy of HALI is often performed targeting iNOS.

In adult rat type II pneumocytes, van Klaveren et al. (1998) found hypoxia exposure increased iNOS mRNA production > 5 fold. Exposure to 100% oxygen for 12 hours may increase the iNOS mRNA by 2.5 folds in the airway epithelial cells from healthy volunteers (Comhair et al., 2000). In a study of Kondrikov et al. (2014), pulmonary artery endothelial cells were exposed to hypoxia (95% O2) for 48 hours, and the decrease in transendothelial electrical resistance (TEER) was found in two phases. In the first phase, the TEER started to decrease at 1 hour and reached the lowest at 3 hours after exposure initiation. In the second phase, the TEER began to decrease at 24 hours after hypoxic exposure and an irreversible decrease was observed after 48-hour exposure, resulting in disruption of monolayer barrier integrity in two phases and apoptosis in the second phase. Moreover, they also found non-selective NOS inhibitor (N[G]-nitro-L-arginine methyl ester, NAME) attenuated the endothelial barrier disruption in both phases; ONOO- scavenger uric acid failed to affect the first phase but ameliorated the second phase of endothelial barrier disruption and apoptosis.

In animal studies, the change in NO production in the lungs is conflicting. In adult rats exposed to hypoxia (for 60 hours), the nitrotyrosine staining exhibited a 2-fold increase as compared to controls (Haddad et al., 1994). Chang et al. (2001) also found hypoxia significantly promoted NO generation via up-regulating iNOS and eNOS, which was also confirmed by the study of Potter et al. (1999). These suggest that excess production in NO following hypoxia exposure is involved in the pathogenesis of HALI. In isolated perfused adult rabbit lungs exposed to 100% oxygen, supplement with L-arginine, a substrate for formation NO via NOS, was found to cause significant edema, which was attenuated by the addition of L-NAME (Nozik et al., 1995). The investigators proposed that H2O2 interacts with NO or one of its oxidized metabolites to result in HALI (Arkovitz et al., 1997). In the study of
Arkovitz et al. (1997), hyperoxia failed to increase serum NO, but resulted in a small but significant increase in NO production in the BALF. In addition, Zhang et al. (2010) reported that osteopontin was able to protect against HALI by inhibiting NOS (iNOS and eNOS) (Table 1).

However, supplement with the non-selective NOS inhibitor NAME is also found to worsen the injury, results in earlier death, decreases tolerance to hyperoxia and abolishes the beneficial effects of a treatment (Pierce et al., 1995; Capellier et al., 1996; Arkovitz et al., 1997; Radomski et al., 1998; Visser et al., 2010). Studies (Sopi et al., 2007; Visser et al., 2010; Ali et al., 2012) also indicate that cGMP decreased after hyperoxia exposure. Thus, some investigators attempt to administer sildenafil to protect the lung against HALI (Ladha et al., 2005; Czovek et al., 2014). In 2-week old rat pups, the ability of endogenous NO to regulate the constriction of central airways was impaired after exposure to ≥ 95% O₂ for 4–6 days (Iben et al., 2000). Contrary to the isolated perfused adult rabbit lungs, studies have shown that hyperoxia up-regulates arginase expression and activity, and supplementation of L-arginine, L-citrulline or arginase blockade restored hyperoxia-induced impairment of relaxation (Sopi et al., 2007; Vadivel et al., 2010; Ali et al., 2012; Grisafi et al., 2012). Other studies also find that some treatments or preventive measures for HALI are able to increase NO production or bioavailability. Ahmed et al. (2011) found transient over-expression of extracellular superoxide dismutase maintained the NO bioavailability, which maintained the cGMP activity and reduced nuclear factor kappa B activation under oxidative stress. Prophylactic treatment with apelin was also found to improve the alveolarization and angiogenesis, increase lung cGMP, reduce pulmonary fibrin deposition, inflammation, and improve the lung morphology, but these beneficial effects were completely abolished in the presence of L-NAME (Visser et al., 2010).

Of note, in the study of Cucchiaro et al. (1999), exposure to 85% oxygen for 24 or 72 hours failed to increase the exhaled NO, exogenous infusion of L-arginine after hyperoxia did not increase the exhaled NO, and immunohistochemistry of the lung showed no nitrotyrosine after hyperoxia exposure. Thus, they concluded that NO was not synthesized in rats exposed to hyperoxia. In the study of Que et al. (1998), male Sprague-Dawley rats were exposed to 100% oxygen for 60 hours, and results showed NOS activity was unchanged and inducible NOS was not induced, but the level of nitrogen oxides (NOx) in the lung decreased by 67%. Potter et al. (1999) found blockade of NOS reduced cGMP in the lung of hyperoxic rat pups, but failed to reverse the pathologic consequences of hyperoxic exposure in these animals.

Of three isoforms of NOS, iNOS is the most extensively studied one in HALI, but there is controversy on the role of iNOS in the pathogenesis of HALI. Bhandari et al. (2012) found NOS2⁻⁻ mice manifested deteriorated alveolar-capillary protein leak and premature death. In the study of Kobayashi et al. (2001), the lung injury was more severe in NOS2⁻⁻ mice than in wild-type mice indicating the anti-inflammatory role of iNOS in HALI. However, in the study of Hesse et al. (2004), the lung injury was more severe in these mice after hyperoxia exposure as compared to iNOS knockout mice, suggesting the pro-inflammatory role of iNOS in HALI. On the above findings, it seems that iNOS has dual roles in the pathogenesis of HALI. Chang et al. (2001) found that hyperoxia significantly promoted NO generation, suggesting that endogenous NO may mediate the hyperoxic pulmonary damage; but over-stimulation of iNOS may lead to the pathogenesis of HALI. Thus, they proposed that NO may have dual roles in pulmonary oxygen toxicity. The dual role of iNOS was also found in the acute asbestos-induced lung injury (Dorger et al., 2002) (Table 2).

In animal studies, most confirm that hyperoxia exposure is able to increase iNOS protein and mRNA expression (Arkovitz et al., 1997; Radomski et al., 1998; Cucchiaro et al., 1999; Potter et al., 1999; Hesse et al., 2004). Radomski et al. (1998) found the activity of Ca²⁺-independent NOS increased by 10 folds after hyperoxia exposure and remained significantly elevated after 14 days of exposure to hyperoxia in the lung of rats. Moreover, there was a time-dependent, biphasic expression (peak at 7 days) of iNOS in the lungs of hyperoxic rats. Thus, some investigators attempt to treat HALI or prevent HALI by targeting iNOS. Yuba et al. (2007) found ONO-1714 was able to attenuate HALI via inhibiting iNOS.

Of note, there is also evidence showing that iNOS seems is not crucial for the pathogenesis of HALI. Although the mice with hyperoxia exposure had a decreased amount of total surfactant, there was no significant difference between wild type mice and iNOS⁻⁻ mice after hyperoxia exposure for 48 hours (Bailey et al., 2002). Bhandari et al. (2012) also found survival was similar in newborn NOS2⁻⁻ and NOS2⁻⁻ mice.

The change in eNOS varies between studies. Steudel et al. (1999) found the expression and activity of both eNOS and iNOS increased in the adult rat lung following hyperoxia exposure, but Arkovitz et al. (1997) found hyperoxia had differential effects on the eNOS and iNOS and hyperoxia was able to decrease eNOS activity. The reduction in eNOS expression was also confirmed by the study of Lu et al. (2015). However, the study of Grisafi et al. (2012) showed eNOS expression increased in hypoxia-treated rats and L-citrulline further increased eNOS expression in the lung, accompanied by the improvement of lung...
Table 1: Studies on the role of nitric oxide (NO) in the pathogenesis of hyperoxic acute lung injury (HALI)

| Study | Animal/cells | Model | Results |
|-------|--------------|-------|---------|
| Haddad et al. (1994) | Adult rats | Exposure to 100% O₂ for 60 hours | The nitrotyrosine staining exhibited a 2-fold increase as compared to controls |
| Nozik et al. (1995) | Isolated perfused rabbit lungs | Ventilation with 95% O₂ | 1.0 mM L-arginine caused significant pulmonary hypertension and edema, which was attenuated by the L-N(G)-nitro-L-arginine methyl ester (NAME) |
| Pierce et al. (1995) | Newborn rats | Exposure to > 95% O₂ | L-NAME was administered to pregnant rats for the final 7 days of gestation and during lactation. The survival rate of L-NAME treated pups when placed in > 95% O₂ at birth was significantly lower than controls from day 4 to 14. Fetal pulmonary artery vasoconstriction was induced by L-NAME |
| Capellier et al. (1996) | Adult rats | Exposure to > 99% O₂ for 36 hours | L-NAME resulted in earlier death. Haematocrit and bronchoalveolar lavage fluid (BALF) protein were also significantly increased in animals exposed to oxygen and receiving L-NAME. The lung water content was slightly decreased by L-NAME. L-NAME failed to affect thiobarbituric acid reactive substances in plasma and lung |
| Arkovitz et al. (1997) | Adult rats | Exposure to 95% O₂ for 3, 4, and 5 days | Hyperoxia did not increase serum NO production, but it resulted in a small and significant increase in NO production in the BALF which was not associated with an induction of whole lung inducible nitric oxide synthases (iNOS). Hyperoxia significantly decreased endothelial constitutive nitric oxide synthase activity. Administration of nitric oxide synthases (NOS) inhibitor worsened the injury |
| Que et al. (1998) | Adult rats | Exposure to 100% O₂ for 60 hours | Hyperoxia significantly increased lung arginase activity and expression, but the lung nitrogen oxides decreased by 67% after hyperoxia |
| Radomski et al. (1998) | 3-day-old rat pups | Exposure to ≥ 95% O₂ for 7 and 14 days | L-NAME in hyperoxic animals reduced lung oedema and epithelial proliferation |
| van Klaveren et al. (1998) | Adult rat type II pneumocytes | Exposure to 60% or 85% O₂ for 48 hours | Exposure to 60% and 85% O₂ decreased nitrite production 2.9-fold and 3.9-fold |
| Potter et al. (1999) | Rat pups | Exposure to > 95% O₂ from day 21 to 29 | Cyclic guanosine monophosphate (cGMP) (NO activity) levels were elevated after hyperoxia exposure, which was attenuated after NOS blockade with either aminoguanidine (AG) or N-nitro-L-arginine (L-NNa). The hyperoxia-induced histologic changes were not altered by NOS blockade with AG or L-NNa |
| Iben et al. (2000) | 13–15-day-old rat pups | Exposure to ≥ 95% O₂ for 4–6 days | Prior blockade of NOS significantly potentiated total lung resistance (RL) response in normoxic animals. The responses of RL to vagal stimulation were increased by hyperoxia, but the NOS blockade induced potentiation of contractile responses was abolished after hyperoxic exposure. NOS blockade potentiated the response of lung elastance in 13- to 15-day-old animals with or without hyperoxic exposure |
| Chang et al. (2001) | 3-day-old preterm rats | ≥ 90% O₂ for 3 or 7 days | Hyperoxia increased NO content in BALF. L-NAME worsened HALI in preterm rats and also had a deleterious effect on the rats exposed to air |
| Ladha et al. (2005) | Newborn rats | Exposure to 95% O₂ | Sildenafil preserved alveolar growth and lung angiogenesis, and decreased pulmonary vascular resistance, right ventricular hypertrophy and medial wall thickness |
| Demchenko et al. (2007) | Adult rats | Exposure to ≥ 98% O₂ for 56 hours | Oxygen at 1 atmosphere absolute increased nitrogen oxides (NOx) and 3-nitrotyrosine in BALF progressively over 56 hours of exposure. |
| Sopi et al. (2007) | Rat pups | Exposure to ≥ 95% O₂ for 7 days | Hyperoxic exposure significantly reduced electrical field stimulation (EFS)-induced relaxation of lung parenchymal strips at 7 and 12 days but not 21 days. NO synthase blockage diminished relaxant responses in room air but not in hyperoxic pups at 12 days. L-arginine restored the relaxation response of hyperoxic strips. cGMP also decreased in strips from hyperoxic pups but was restored by L-arginine. Hyperoxia significantly increased arginase activity of lung parenchymal strips |
| Vadivel et al. (2010) | Newborn rats | Exposure to 95% O₂ from birth through postnatal day (P) 14 | L-citrulline prevents HALI and pulmonary hypertension in newborn rats |
| Visser et al. (2010) | Neonatal rats | Exposure to 100% O₂ | Prophylactic treatment with apelin improved HALI and increased lung cGMP levels, which were completely absent in the presence of L-NAME |
| Ahmed et al. (2011) | Neonatal rabbits | Exposure to 95% O₂ for 7 days | cGMP increased significantly in human extracellular superoxide dismutase-transfected neonatal rabbit lung tissues after hyperoxia exposure for 3 and 7 days |
| Ali et al. (2012) | Rat pups | Exposure to ≥ 50% O₂ for 7 days | In bethanechol-preconstricted lung parenchymal strips, hyperoxia significantly reduced relaxation, which was restored by L-arginine or arginase blockade. Expression and activity of arginase in airway epithelium were increased in response to hyperoxia but reduced by arginase blockade. EFS-induced production of NO was decreased in hyperoxia-exposed airway smooth muscle and restored by arginase blockade |
| Grisafi et al. (2012) | Newborn rats | Exposure to 60% O₂ for 14 days | L-citrulline was effective to improve alveolar and vascular growth in oxygen-induced pulmonary damage |
| Czovek et al. (2014) | 28-day-old rats | Exposure to ≥ 95% O₂ for 72 hours | Sildenafil was able to protect the lung against hyperoxia-induced bronchial hyperreactivity via preserving normal end-expiratory lung volume, inhibiting airway inflammation and preserving the physiological lung structure |
| Kondrikov et al. (2014) | Pulmonary artery endothelial cells | Exposure to 95% O₂ for 48 hours | Hyperoxia caused the disruption of transepithelial electrical resistance (TEER) in two phases and the apoptosis in the second phase. NOS inhibitor attenuated the TEER disruption in both phases; Peroxynitrite scavenger uric acid did not affect the first phase but ameliorated the second phase of TEER disruption and apoptosis |
Table 2: Studies on the role of inducible nitric oxide synthases (iNOS) and endothelial nitric oxide synthases (eNOS) in the pathogenesis of hyperoxic acute lung injury (HALI)

| Study                        | Animal/cells                          | Model                                | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|------------------------------|---------------------------------------|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Que et al. (1998)            | Adult rats                            | Exposure to 100% O₂ for 60 hours     | Nitric oxide synthases (NOS) activity and iNOS remained unchanged after hyperoxia exposure                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Radomski et al. (1998)       | 3-day-old rat pups                    | Exposure to ≥ 95% O₂ for 7 and 14 days | Hyperoxia caused a decrease in Ca²⁺-dependent NOS activity, which was associated with increased eNOS protein expression. Hyperoxia caused 10-fold increase in the activity of Ca²⁺-independent NOS which remained significantly elevated after 14-day exposure                                                                                                                                                                                                                                                               |
| van Klaveren et al. (1998)   | Adult rat type II pneumocytes          | Exposure to 60% or 85% O₂ for 48 hours | Exposure to 85% O₂ increased iNOS mRNA production 5.4-fold                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Cucchiaro et al. (1999)      | Adult rats                            | Exposure to 85% O₂ for 24 and 72 hours | Hyperoxia induced iNOS expression in the lung, but exhaled nitric oxide (eNO) was not elevated by hyperoxia and exogenous L-arginine after hyperoxia did not increase eNO. Nitrotyrosine was not found in the hyperoxic group after NO was scavenged by oxygen radicals to form peroxynitrite                                                                                                                                                                                                                                                                   |
| Potter et al. (1999)         | Rat pups                              | Exposure to > 95% O₂ from day 21 to 29 | Hyperoxia significantly up-regulated the expression of iNOS and eNOS in inflammatory cells and epithelia in the lungs of preterm rats. Over-stimulation of iNOS contributes to the pathogenesis of HALI                                                                                                                                                                                                                                                                                                                                                      |
| Steudel et al. (1999)        | Adult rats                            | Exposure to > 87% O₂ for 28 days     | NOS (NOSII and NOSIII) expression and activity significantly increased in hyperoxia, and NOS III expression increased selectively in vascular endothelial cells, while both NOS isoforms were expressed by the pulmonary alveolar macrophages                                                                                                                                                                                                                                                                                                           |
| Comhair et al. (2000)        | Human airway epithelial cells from healthy volunteers | Exposure to 100% O₂ for 12 hours  | Hyperoxia increased NOS2 mRNA in airway epithelial cells by 2.5-fold                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |
| Chang et al. (2001)          | 3-day-old preterm rats                | ≥ 90% O₂ for 3 or 7 days             | Hyperoxia increased iNOS airway and alveolar epithelial cells. iNOS expression after 7-day exposure was stronger than after 3-day exposure. Shortly after 7-day exposure, stronger immunostaining for eNOS in airway epithelial was seen. L-N[(G)-nitro-L-arginine methyl ester worsened HALI in preterm rats and also had a deleterious effect on the rats exposed to air                                                                                                                                                                                                |
| Kobayashi et al. (2001)      | Adult mice                            | Exposure to > 98% O₂ for 72 hours    | Lung injury was more severe in iNOS-deficient mice and associated with increased polymorphonuclear leukocytes in bronchoalveolar lavage fluid (BALF). iNOS mRNA expression increased in the lungs of wild-type hyperoxic mice. Nitrotyrosine was expressed in both wild-type and iNOS-deficient mice in hyperoxia                                                                                                                                                                                                                     |
| Bailey et al. (2002)         | Adult mice                            | Exposure to > 90% O₂ for 48 hours    | Hyperoxia decreased total surfactant, but there was no significant difference between wild-type and iNOS⁻ mice                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| Hesse et al. (2004)          | 12–16 weeks old mice                  | 60% O₂ or > 95% O₂ for 72 hours      | Hyperoxia induced a significant increase in total cell count, protein concentration, lactate dehydrogenase activity, lipid peroxidation, and tumor necrosis factor-α concentration in the BALF as well as a higher binding activity of nuclear factor kappa B and activator protein-1 as compared to iNOS knockout mice                                                                                                                                                                                                                           |
| Yuba et al. (2007)           | Adult mice                            | Exposure to > 98% O₂ for 72 hours    | ONO markedly inhibited iNOS protein expression and nitrotyrosine production in lung homogenates and attenuated lung injury                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Zhang et al. (2010)          | Adult mice                            | Exposure to > 95% O₂ for 24-72 hours | Hyperoxia exposure significantly increased mRNA and protein expressions of iNOS and eNOS in osteopontin knockout mice than their matched wild-type mice                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Bhandari et al. (2012)       | Adult and newborn mice                | Newborn mice exposed to 100% O₂ since PN1 with alternation in hyperoxia and room air every 24 hours; adult mice exposed to 100% O₂ for 60 hours | NOS²⁻ animals manifest exaggerated alveolar-capillary protein leak and premature death. Survival was similar in adult NOS³⁻ and NOS³⁻ and NB NOS²⁻ and NOS²⁻ mice, respectively                                                                                                                                                                                                                                                                                                                                                                 |
| Grisafi et al. (2012)        | Newborn rats                          | Exposure to 60% O₂ for 14 days       | Hyperoxia exposure increased eNOS protein expression and L-citrulline in combination with hyperoxia further increase eNOS protein expression                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| Saric et al. (2014)          | Female and male CBA/H mice            | Exposure to 95% O₂ for 48 hours      | Hyperoxia significantly up-regulated eNOS protein expression in females, but not in males. However, eNOS mRNA expression increased significantly after hyperoxia exposure in both males and females. Females showed better survival, were resistance to hyperoxia and had more efficient defense systems                                                                                                                                                                                                                                                                                                                                                                                    |
| Lu et al. (2015)             | Neonatal rats                         | Exposure to 85% O₂ for 28 days       | Hyperoxia exposure led to low expression of eNOS. Inhaled nitric oxide and transplanted endothelial progenitor cells increased eNOS protein expression
injury, suggesting that eNOS is protective on HALI. Saric et al. (2014) found eNOS was significantly up-regulated in hyperoxia-exposed groups of both sexes, and difference between sexes was insignificant but eNOS protein level was significantly up-regulated in hyperoxia-exposed females, while in males the increase in eNOS protein did not reach significance due to a large variation among individual mice. The eNOS expression after hyperoxic exposure was also confirmed in the study of Potter et al. (1999), but the increase in eNOS was lower than in iNOS after hyperoxia exposure. Radomski et al. (1998) found hyperoxia increased the protein expression of eNOS in the lung after hyperoxia, and the eNOS expression reduced with the prolongation of hyperoxia exposure, but hyperoxia decreased Ca²⁺-dependent NOS activity. However, Bhandari et al. (2012) found the survival was similar in adult NOS3+/+ and NOS3-/- mice.

In acute pulmonary oxygen toxicity following hyperbaric oxygen exposure, Demchenko et al. (2007) proposed that extrapulmonary, neurogenic events predominated in the pathogenesis of lung injury, and nNOS activity drove the lung injury by regulating the output of central autonomic pathways.

**TREATMENT OF HALI WITH EXOGENOUS NO**

Low dose iNO has been approved by the US Food and Drug Administration (FDA) for the treatment of persistent pulmonary hypertension of the newborn (Ballard et al., 2006b) and this treatment is safe without adverse short or long term sequelae. The therapeutic use of iNO has expanded to older patients with congenital heart disease and ARDS. However, whether exogenous NO is protective or harmful is still unclear when it is administered in combination with hyperoxia (Rieger-Fackeldey and Hentschel, 2008).

**Effects of iNO on HALI**

In *vivo*, Turanlahti et al. (2000) found 6-hour exposure to NO at 40 ppm alone induced free radical-mediated lung injury, but 40 ppm NO in the presence of hyperoxia significantly attenuated free radical-mediated effects in the lungs compared to hyperoxia or 40 ppm NO alone. In the study of Nelin et al. (2003), NO at 100 ppm alone led to greater salicylate hydroxylation, but 100 ppm NO resulted in less salicylate hydroxylation than either did individually when it was administered during hyperoxia exposure; the production of hydrolytic radical (OH') and/or ONOO⁻ in the lung during iNO was dependent on the ratio of NO to oxygen (10 ppm and 100 ppm NO was used in this study in combination with oxygen at different concentrations). However, in the study of Youssef et al. (1999), iNO at 50 ppm did not improve HALI. In the study of Gries et al. (2000), NO exposure for 5 days alone had no notice-

able respiratory effects and could not cause pulmonary dysfunction, but short-term exposure (≤ 5 days) to NO/O₂ was able to delay the onset of respiratory distress and neither deteriorated nor alleviated lung dysfunction as compared to oxygen exposure alone. In neonatal mice exposed to > 95% oxygen, the mortality occurred earlier in pups exposed to the mixture of > 95% oxygen and NO than in those with hyperoxia exposure alone (Stenger et al., 2010). The Ekekezie’s group (Ekekezie et al., 2000a) found 5-day exposure to NO (50 ppm) significantly increased lung collagen content, but this effect appeared potentially reversible; in contrast, hyperoxia exposure with or without NO resulted in pulmonary matrix degradation and increased lung collagen content. The authors proposed that NO could potentially induce pulmonary fibrosis. In Fischer 344 rats, hyperoxia failed to induce significant injury to mitochondrial DNA (mtDNA), whereas iNO in the presence of hyperoxia (> 95% O₂) caused mtDNA damage in the lung, but the lesions were rapidly repaired during recovery, and the ratio of pulmonary mtDNA to genomic DNA was the same between treatment groups (Lightfoot et al., 2004).

Couroucli et al. (2006) found newborn rats exposed to iNO at 40 ppm in the presence of hyperoxia were more susceptible to lung injury than rat pups exposed to hyperoxia alone or iNO alone, but animals with 20 ppm iNO exposure in the presence of hyperoxia did not elicit lung damage. This suggests that the effects of iNO on HALI are NO dose dependent. In the study of Garat et al. (1997), results suggested that, depending on its concentration, iNO could either reduce or increase the early consequences of HALI (10 ppm iNO attenuated the increases in thiobarbituric acid reactive substances (TBARS) and wet to dry lung weight ratio [Qw/Qd] of the lungs, did not affect the alveolar barrier impermeability to protein, and improved alveolar liquid clearance, but 100 ppm iNO had no effect on the increased TBARS and Qw/Qd but elevated vascular permeability to protein). ter Horst et al. (2007) also investigated the effects of iNO at different concentrations. Results showed, in the presence of 100% oxygen exposure, continuous exposure to NO at 8.5 and 17 ppm NO reduced fibrin deposition by 1.6-fold and 4.3-fold, respectively; the survival in 17-ppm NO group was prolonged as compared to animals with oxygen exposure alone; the reduction in pro-inflammatory cytokines after 17-ppm NO-O₂-exposure was more obvious than after 8.5-ppm NO-O₂-exposure; the histology was also improved after NO-O₂-exposure. In addition, iNO at 50 ppm alone was also found to decrease intracellular oxidant production, but iNO during hyperoxia did not impact lung neutrophil accumulation, instead increased lung apoptosis and prevented the increase in SOD and catalase activity during hyperxia, potentially increasing injury (Ekekezie
Mechanisms underlying the protective effects of iNO on HALI

Although there is controversy on the protective effects of iNO on HALI, a majority of studies confirm that iNO may exert protective effects (improvement of pulmonary angiogenesis, lung alveolarization, distal lung growth and pulmonary function) on HALI via different mechanisms in rodents, pigs and primates (Rieger-Fackeldey and Hentschel, 2008).

Youssef et al. (1999) found 50 ppm of iNO reduced the expression of Na, K-ATPase which constituted a part of the cellular defense mechanism against oxygen toxicity (vascular permeability). iNO may also inhibit inflammation, reduce fibrin deposition (Lightfoot et al., 2004), nitrate stress (Stenger et al., 2010) and plasminogen activator inhibitor-1 (Chen et al., 2015), decrease leukocyte trafficking (Ekekezie et al., 2001; Rose et al., 2010), suppress cell apoptosis (Howlett et al., 1999), induce marked pulmonary vasodilatation (Mourani et al., 2004), elevate angiogenesis (Lin et al., 2005), improve lung volume (Ballard et al., 2006a) and restore distal lung growth (Lin et al., 2005).

iNO in hyperoxia was also found to improve the hyperoxia-induced surfactant abnormality in primates (Ballard et al., 2006a) and preterm rabbits (Issa et al., 1999), but this was not found in human alveolar type II cells (Johnston et al., 2010). Couroucli et al. (2006) proposed that the hyperxia induced expression of CYP1A1 was protective against HALI, and the maintenance of pulmonary CYP1A1 induction by 20 ppm iNO in the presence of hyperoxia was related to the protective effects of iNO on HALI in newborn rats, but Cotton et al. (2006) found the protective effects of iNO had no relationship with CYP activity in lambs exposed to > 95% O2. In rat lungs, Gong et al found inhaled NO alleviated hyperoxia suppressed phosphatidylcholine synthesis in rats with endotoxin-induced injury (Gong et al., 2006), but the results of Hu’s study (Hu et al., 2007) showed iNO attenuated hyperoxic injury without altering phosphatidylcholine synthesis.

Different uses of iNO

In the above studies, iNO is usually administered during the hyperoxia exposure, which implies that iNO is employed for the prevention of HALI. In a study of Waldow et al. (2008), they found pre-conditioning by inhaled NO (15 ppm, 10 minutes) was able to prevent hyperoxic and ischemia/reperfusion injury in adult rat lungs. In addition, Lu et al. (2015) investigated the protective effects of inhaled NO in combination with endothelial progenitor cells transplantation on HALI, and results showed this was able to improve lung alveolar and vascular structure in neonatal rats with prolonged hyperoxia exposure.

Although a lot studies have investigated the roles of NO and NOS in the pathogenesis of HALI, the available findings are still conflicting. This may be ascribed to the differences in the animal model, hyperoxia exposure protocol, time points of sample collection, methods used for the detections and the gender and age of animals used in experiments. In addition, in respect of iNO treatment of HALI, the dose of NO varies significantly and ranges from 5 ppm to 100 ppm in animal studies although the concentration of NO used in clinical practice has been determined, which may cause the discrepancy in the findings between studies. Thus, more studies are warranted to confirm the roles of NO and different iNOS in the pathogenesis of HALI and the therapeutic effects of iNO on HALI.

Author contributions
XJS designed this paper; KL and JZ collected literatures; PXZ, CHH, and WWL wrote the paper. All the authors approved the final version of the paper for publication.

Conflicts of interest
There is no conflict of interest in this paper.

REFERENCES
Ahmed MN, Codipilly C, Hogg N, Auten RL (2011) The protective effect of overexpression of extracellular superoxide dismutase on nitric oxide bioavailability in the lung after exposure to hyperoxia stress. Exp Lung Res 37:10-17.
Ali NK, Jafri A, Sopi RB, Prakash YS, Martin RJ, Zaidi SI (2012) Role of arginase in impairing relaxation of lung parenchyma of hyperoxia-exposed neonatal rats. Neonatology 101:106-115.
Allen BW, Demchenko IT, Piantadosi CA (2009) Two faces of nitric oxide: implications for cellular mechanisms of oxygen toxicity. J Appl Physiol (1985) 106:662-667.
Arkowitz MS, Wispe JR, Garcia VF, Szabo C (1996) Selective inhibition of the inducible isoform of nitric oxide synthase prevents pulmonary transvascular flux during acute endotoxia. J Pediatr Surg 31:1009-1015.
Arkowitz MS, Szabo C, Garcia VF, Wong HR, Wispe JR (1997) Differential effects of hyperoxia on the inducible and constitutive isoforms of nitric oxide synthase in the lung. Shock (Augusta, Ga) 7:345-350.
Bailey TC, Cavanagh C, Mehta S, Lewis JF, Veldhuizen RA (2002) Sepsis and hyperoxia effects on the pulmonary surfactant system in wild-type and iNOS knockout mice. Eur Respir J 20:177-182.
Demchenko IT, Welty-Wolf KE, Allen BW, Piantadosi CA (2007) Similar but not the same: normobaric and hyperbaric pulmonary oxygen toxicity, the role of nitric oxide. Am J Physiol Lung Cell Mol Physiol 293:L229-238.

Bogdan C (2015) Nitric oxide synthase in innate and adaptive immunity: an update. Trends Immunol 36:161-178.

Bredt DS (1999) Endogenous nitric oxide synthesis: biological functions and pathophysiology. Free Radic Res 31:577-596.

Bult H, Boeckxstaens GE, Pelekmans PA, Jordaeus FH, Van Maeckeke YM, Herman AG (1990) Nitric oxide as an inhibitory nonadrenergic non-cholinergic neurotransmitter. Nature 345:346-347.

Burnett AL (2008) Molecular pharmacotherapeutic targeting of PDE5 for preservation of penile health. J Androl 29:3-14.

Capellier G, Maupoil V, Boillot A, Kantelip JP, Rochette L, Regnard J, Barale F (1996) L-NAME aggravates pulmonary oxygen toxicity in rats. Eur Respir J 9:2531-2536.

Chang L, Ma L, Zhang X, Chen Y (2001) The role of nitric oxide in hyperoxic lung injury in premature rats. Tongji Yike Daxue Xuebao 21:78-81.

Chen Y, Lu ZJ, Yang Y, Lu GP, Chen WM, Zhang LE (2015) Suppression of plasminogen activator inhibitor-1 by inhaled nitric oxide attenuates the adverse effects of hyperoxia in a rat model of acute lung injury. Thromb Res 136:131-138.

Comhair SA, Thomassen MJ, Erzurum SC (2000) Differential induction of extracellular glutathione peroxidase and nitric oxide synthase 2 in airways of healthy individuals exposed to 100% O(2) or cigarette smoke. Am J Respir Cell Mol Biol 23:350-354.

Cotton RB, Sundell HW, Zeldin DC, Morrow JD, Roberts LJ, Hazinski TA, Law AB, Steele S (2006) Inhaled nitric oxide attenuates hyperoxic lung injury in newborn guinea pigs. Am J Physiol Regul Integr Comp Physiol 297:R1525-1530.

Grisafi D, Tassone E, Dedda H, Onisto M, Milan A, Macchi V, De Caro R, Fassina A, Bordigato MA, Chiandetti L, Filippone M, Zaramella P (2012) L-citrulline reduces alveolar and vascular derangement in a rat model of moderate hyperoxia-induced lung injury. Lung 190:419-430.

Guo FH, De Raeye HR, Rice TW, Stuehr DJ, Thunnissen FB, Erzurum SC (1995) Continuous nitric oxide synthesis by inducible nitric oxide synthase in normal human airway epithelium in vivo. Proc Natl Acad Sci U S A 92:7809-7813.

Guo FH, Comhair SA, Zheng S, Dweik RA, Eissa NT, Thomassen MJ, Calhoun W, Erzurum SC (2000) Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis. J Immunol 164:5970-5980.

Haddad JY, Pataki G, Hu P, Galliani C, Beckman JS, Matalon S (1994) Quantitation of nitrotyrosine levels in lung sections of patients and animals with acute lung injury. J Clin Invest 94:2407-2413.
Hamad AM, Knox AJ (2001) Mechanisms mediating the antiproliferative effects of nitric oxide in cultured human airway smooth muscle cells. FEBS Lett 506:91-96.

Hamid Q, Springall DR, Riveros-Moreno V, Chanez P, Howarth P, Redington A, Bousquet J, Godard P, Holgate S, Polak JM (1993) Induction of nitric oxide synthase in asthma. Lancet 342:1510-1513.

Hampson N, Atik D (2003) Central nervous system oxygen toxicity during routine hyperbaric oxygen therapy. Undersea Hyperb Med 30:147-153.

Hesse AK, Dorger M, Kupatt C, Krombach F (2004) Proinflammatory role of inducible nitric oxide synthase in acute hyperoxic lung injury. Respir Res 5:11.

Howlett CE, Hutchison JS, Veinot JP, Chiu A, Merchant P, Fliss H (1999) Inhaled nitric oxide protects against hyperoxia-induced apoptosis in rat lungs. Am J Physiol 277:L596-605.

Hu Q, Manaenko A, Guo Z, Huang L, Tang J, Zhang JH (2015) Hyperbaric oxygen therapy for post concussion symptoms: issues may affect the results. Med Gas Res 5:10.

Hu X, Guo C, Sun B (2007) Inhaled nitric oxide attenuates hyperoxic and inflammatory injury without alteration of phosphatidylcholine synthesis in rat lungs. Pulm Pharmacol Ther 20:75-84.

Iben SC, Dreshaj IA, Farver CF, Haxhiu MA, Martin RJ (2000) Role of endogenous nitric oxide in hyperoxia-induced airway hyperreactivity in maturing rats. J Appl Physiol (1985) 89:1205-1212.

Issa A, Lappalainen U, Kleinman M, Bry K, Hallman M (1999) Inhaled nitric oxide decreases hyperoxia-induced surfactant abnormality in preterm rabbits. Pediatr Res 45:247-254.

Johnston LC, Gonzales LW, Lightfoot RT, Guttentag SH, Ischiropoulos H (2010) Opposing regulation of human alveolar type II cell differentiation by nitric oxide and hyperoxia. Pediatr Res 67:521-525.

Kallett RH, Matthay MA (2013) Hyperoxic acute lung injury. Respir Care 58:123-141.

Kobayashi H, Hataishi R, Mitsufuji H, Tanaka M, Jacobson M, Tomita T, Zapol WM, Jones RC (2001) Antiinflammatory properties of inducible nitric oxide synthase in acute hyperoxic lung injury. Am J Respir Cell Mol Biol 24:390-397.

Kobzik L, Breit DS, Lowenstein CJ, Drazen J, Gaston B, Sugarbaker D, Stamler JS (1993) Nitric oxide synthase in human and rat lungs: immunocytochemical and histochemical localization. Am J Respir Cell Mol Biol 9:371-377.

Kondrikov D, Gross C, Black SM, Su Y (2014) Novel peptide for attenuation of hyperoxic-induced disruption of lung endothelial barrier and pulmonary edema via modulating peroxynitrite formation. J Biol Chem 289:33355-33363.

Ladha F, Bonnet S, Eaton F, Hashimoto K, Korbutt G, Thebaud B (2005) Sildenafil improves alveolar growth and pulmonary hypertension in hyperoxia-induced lung injury. Am J Resp Crit Care Med 172:750-756.

Lane C, Knight D, Burgess S, Franklin P, Horak F, Legg J, Moeller A, Stick S (2004) Epithelial inducible nitric oxide synthase activity is the major determinant of nitric oxide concentration in exhaled breath. Thorax 59:757-760.

Lee YC, Cheon KT, Lee HB, Kim W, Rhee YK, Kim DS (2000) Gene polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in patients with asthma. Allergy 55:959-963.

Li L, Zuo Y, He J (2014) Preconditioning crush increases the survival rate of motor neurons after spinal root avulsion. Neural Regen Res 9:540-548.

Lightfoot RT, Khou S, Ischiropoulos H (2004) Transient injury to rat lung mitochondrial DNA after exposure to hyperoxia and inhaled nitric oxide. Am J Physiol Lung Cell Mol Physiol 286:L23-29.

Lin YJ, Markham NE, Balasubramaniam V, Tang JR, Maxey A, Kinsella JP, Abman SH (2005) Inhaled nitric oxide enhances distal lung growth after exposure to hyperoxia in neonatal rats. Pediatr Res 58:22-29.

Liu C, Cui Z, Wang S, Zhang D (2014) CD93 and GIPC expression and localization during central nervous system inflammation. Neural Regen Res 9:1995-2001.

Lu A, Sun B, Qian L (2015) Combined iNO and endothelial progenitor cells improve lung alveolar and vascular structure in neonatal rats exposed to prolonged hyperoxia. Pediatr Res 77:784-792.

Luhrs H, Papadopoulos T, Schmidt HH, Menzel T (2002) Type I nitric oxide synthase in the human lung is predominantly expressed in capillary endothelial cells. Respir Physiol 129:367-374.

Lv R, Zheng J, Ye Z, Sun X, Tao H, Liu K, Li R, Xu W, Liu W, Zhang R (2014) Advances in the therapy of hyperoxia-induced lung injury: findings from animal models. Undersea Hyperb Med 41:183-202.

Maestrelli P, Paska C, Saetta M, Turato G, Nowicki Y, Monti S, Formichi B, Miniati M, Fabbri LM (2003) Decreased haem oxygenase-1 and increased inducible nitric oxide synthase in the lung of severe COPD patients. Eur Respir J 21:971-976.

Mourani PM, Ivy DD, Gao D, Abman SH (2004) Pulmonary vascular effects of inhaled nitric oxide and oxygen tension in broncho-pulmonary dysplasia. Am J Resp Crit Care Med 170:1006-1013.

Nelin LD, Morrisey JF, Effros RM, Dawson CA, Schaipira RM (2003) The effect of inhaled nitric oxide and oxygen on the hydroxylation of salicylate in rat lungs. Pediatr Res 54:337-343.

Nichols CW, Lamberts C (1969) Effects of high oxygen pressures on the eye. N Engl J Med 281:25-30.

Nozik ES, Huang YC, Piantadosi CA (1995) L-arginine enhances injury in the isolated rabbit lung during hyperoxia. Respir Physiol 100:63-74.

Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87:315-424.

Patel HI, Belvisi MG, Donnelly LE, Yacoub MH, Chung KF, Mitchell JA (1999) Constitutive expressions of type I NOS in human airway smooth muscle cells: evidence for an antiproliferative role. FASEB J 13:1810-1816.

Pierce MR, Voelker CA, Sosenko IR, Bustamante S, Oli ster SM, Zhang XJ, Clark DA, Miller MJ (1995) Nitric oxide synthase inhibition decreases tolerance to hyperoxia in newborn rats. Media tors Inflamm 4:431-436.

Potter CF, Kuo NT, Farver CF, McMahon JT, Chang CH, Agami FH, Haxhiu MA, Martin RJ (1999) Effects of hyperoxia on nitric oxide synthase expression, nitric oxide activity, and lung injury in rat pups. Pediatr Res 45:8-13.

Que LG, Kantrow SP, Jenkinson CP, Piantadosi CA, Huang YC (1998) Induction of arginase isoforms in the lung during hyperoxia. Am J Physiol 275:L96-102.

Radomski A, Sawicki G, Olson DM, Radomski MW (1998) The role of nitric oxide and metalloproteinases in the pathogenesis of hyperoxia-induced lung injury in newborn rats. Br J Pharmacol 125:1455-1462.
Raghuram N, Fortenberry JD, Owens ML, Brown LA (1999) Effects of exogenous nitric oxide and hyperoxia on lung fibroblast viability and DNA fragmentation. Biochem Biophys Res Commun 262:685-691.

Redington AE (2006) Modulation of nitric oxide pathways: therapeutic potential in asthma and chronic obstructive pulmonary disease. Eur J Pharmacol 533:263-276.

Redington AE, Howarth PH (1997) Airway wall remodelling in asthma. Thorax 52:310-312.

Redington AE, Meng QH, Springall DR, Evans TJ, Creminion C, Maclouf J, Holgate ST, Howarth PH, Polak JM (2001) Increased expression of inducible nitric oxide synthase and cyclo-oxygenase-2 in the airway epithelium of asthmatic subjects and regulation by corticosteroid treatment. Thorax 56:351-357.

Ricciardolo FL, Timmers MC, Gepetti P, van Schadewijk A, Brahim JJ, Sont JK, de Gouw HW, Hiemstra PS, van Kriecken JH, Sterk PJ (2001) Allergen-induced impairment of bronchoprotective nitric oxide synthesis in asthma. J Allergy Clin Immunol 108:198-204.

Rieger-Fackeldey E, Hentschel R (2008) Bronchopulmonary dysplasia and early prophylactic inhaled nitric oxide in preterm infants: current concepts and future research strategies in animal models. J Perinat Med 36:442-447.

Rose MJ, Stenger MR, Joshi MS, Welty SE, Bauer JA, Nelin LD (2010) Inhaled nitric oxide decreases leukocyte trafficking in the neonatal mouse lung during exposure to >95% oxygen. Pediatr Res 67:244-249.

Saric A, Sobocanec S, Safranko Z, Popovic-Hadzija M, Aralica G, Korolija M, Kusic B, Balog T (2014) Female headstart in resistance to hyperoxia-induced oxidative stress in mice. Acta Biochim Pol 61:801-807.

Shaul PW, North AJ, Wu LC, Wells LB, Brannon TS, Lau KS, Michel T, Margraf LR, Star RA (1994) Endothelial nitric oxide synthase is expressed in cultured human bronchiolar epithelium. J Clin Invest 94:2231-2236.

Sittipunt C, Steinberg KP, Ruzinski JT, Myles C, Zhu S, Goodman RB, Hudson LD, Matalon S, Martin TR (2001) Nitric oxide and nitrotyrosine in the lungs of patients with acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol 279:L1029-1036.

Sopri RB, Haxhiu MA, Martin RJ, Dreshaj IA, Kamath S, Zaidi SI (2007) Disruption of NO-cGMP signaling by neonatal hyperoxia impairs relaxation of lung parenchyma. Am J Physiol Lung Cell Mol Physiol 293:L35-44.

Stenger MR, Rose MJ, Joshi MS, Rogers LK, Chicoine LG, Bauer JA, Nelin LD (2010) Inhaled nitric oxide prevents 3-nitrotyrosine formation in the lungs of neonatal mice exposed to >95% oxygen. Lung 188:217-227.

Steudel W, Watanabe M, Dikranian K, Jacobson M, Jones RC (1999) Expression of nitric oxide synthase isoforms (NOS II and NOS III) in adult rat lung in hyperoxic pulmonary hypertension. Cell Tissue Res 295:317-329.

Stoller KP (2015) All the right moves: the need for the timely use of hyperbaric oxygen therapy for treating TBI/CTE/PTSD. Med Gas Res 5:7.

Storme L, Riou Y, Dubois A, Fialdes P, Jaillard S, Klosowski S, Dupuis B, Lequien P (1999) Combined effects of inhaled nitric oxide and hyperoxia on pulmonary vascular permeability and lung mechanics. Crit Care Med 27:1168-1174.

Stower Horst SA, Walther FJ, Poorthuis BJ, Hiemstra PS, Wagenaar GT (2007) Inhaled nitric oxide attenuates pulmonary inflammation and fibrin deposition and prolongs survival in neonatal hyperoxic lung injury. Am J Physiol Lung Cell Mol Physiol 293:L35-44.

Thomson L, Paton J (2014) Oxygen toxicity. Paediatr Respir Rev 15:120-123.

Turanlahti M, Pesonen E, Lassus P, Andersson S (2000) Nitric oxide and hyperoxia in oxidative lung injury. Acta Paediatr 89:966-970.

Vadivel A, Aschner JL, Rey-Purra GJ, Magarik J, Zeng H, Summar M, Eaton F, Thebaud B (2010) L-citrulline attenuates arrested alveolar growth and pulmonary hypertension in oxygen-exposed lung injury in newborn rats. Pediatr Res 68:519-525.

van Klaveren RJ, Roelant C, Boogaerts M, Pype JL, Demedts M, Nemery B (1998) Protective effects of the lazaroid U-74389G against hyperoxia in rat type II pneumocytes. Pulm Pharmacol Ther 11:23-30.

van Straaten JF, Postma DS, Coers W, Noordhoek J, Kauffman HF, Timens W (1998) Macrophages in lung tissue from patients with pulmonary emphysema express both inducible and endothelial nitric oxide synthase. Mod Pathol 11:648-655.

Visser YP, Walther FJ, Laghmani el H, Laarse A, Wagenaar GT (2010) Apelin attenuates hyperoxic lung and heart injury in neonatal rats. Am J Resp Crit Care Med 182:1239-1250.

Waldow T, Witt W, Ulmer A, Janke A, Alexiou K, Matschke K (2008) Preconditioning by inhaled nitric oxide prevents hyperoxic and ischemia/reperfusion injury in rat lungs. Pulm Pharmacol Ther 21:418-429.

Ward JK, Barnes PJ, Springall DR, Abelli L, Tadjkarimi S, Yacoub MH, Polak JM, Belvisi MG (1995) Distribution of human iNANC bronchodilator and nitric oxide-immunoreactive nerves. Am J Respir Cell Mol Biol 13:175-184.

Weaver J, Liu KJ (2015) Does normobaric hyperoxia increase oxidative stress in acute ischemic stroke? A critical review of the literature. Med Gas Res 5:11.

Yanamandra K, Buggs PB, Thurmon TF, Lewis D, Bocchini JA, Jr., Dhanireddy R (2005) Novel allele of the endothelial nitric oxide synthase gene polymorphism in Caucasian asthmatics. Biochem Biophys Res Commun 335:545-549.

Youssef JA, Thibeault DW, Rezaiekhahiligh MH, Mabry SM, Norberg MI, Truong WE (1999) Influence of inhaled nitric oxide and hyperoxia on Na, K-ATPase expression and lung edema in newborn piglets. Biol Neonate 75:199-209.

Yuba T, Nagata K, Yamada T, Osugi S, Kuwahara H, Iwasaki Y, Band O, Naito Y, Yoshikawa T, Marunaka Y (2007) A novel potent inhibitor of inducible nitric oxide synthase. Mod Pathol 11:23-30.

Zhang XF, Liu S, Zhou Y, Zhu GF, Foda HD (2010) Osteopontin effects of exogenous nitric oxide and hyperoxia on lung fibroblast viability and DNA fragmentation. Thorax 56:351-357.

Zhu S, Ware LB, Geiser T, Matthay MA, Matalon S (2001) Inhaled nitric oxide attenuates pulmonary inflammation and fibrin deposition in the pulmonary edema fluid of patients with acute lung injury. Am J Physiol Lung Cell Mol Physiol 279:L35-44.