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CHAPTER 12
The Physiology and Pathophysiology of Nitric Oxide in the Lung

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1. Nitric Oxide: Biosynthesis and Sources in the Lung

The free radical nitric oxide (NO) is generated by the five electron oxidation of a guanidino nitrogen from L-arginine by a group of enzymes termed NO synthases (NOS). In the past decade, it has become clear that this simple molecular species has a remarkable chemical versatility, allowing it to participate in a variety of physiological and pathophysiological cellular processes in the lung [1]. NO has been characterized, for example, as a neurotransmitter [2], a second messenger [3, 4], a paracrine hormone [5, 6], and a cytotoxin [3, 7]. Accordingly, the production, distribution and fate of NO

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are tightly regulated [8–10]. Derangement of this balance may result in a state of NO deficiency or excess [11], manifested by inflammation [12–14], hypotension [15], transplant rejection [16], macromolecular epithelial hyperpermeability [17], hypertension [18], smooth muscle hyperplasia [19, 20] and ischemia/reperfusion injury [21]. Currently, clinical investigations are underway in which the level of tissue NO is pharmacologically manipulated by exogenous delivery of NO in its gaseous form or via inhibition of NO synthases. The proper application of these approaches in the treatment of pulmonary disease, as well as an understanding of their anticipated side-effects, necessitates an understanding of the physiological and pathophysiologic role of NO in the lung [3]. This review focuses on the role of nitric oxide in the lung and discusses the potential therapeutic options in the management of NO-related disease.

Data in support of pulmonary NO synthesis are derived from both clinical studies and experimental models. Direct evidence of NO formation has been provided by immunohistochemical identification of NO synthases and by measurement of NO production or NOS activity from exhaled gas [22–25], pulmonary tissue [26] and isolated cells [27, 28]. Indirect evidence of NO synthesis has been obtained from observations of physiological and *ex vivo* alterations resulting from exogenous provision of NO or pharmacological ablation of endogenous NO formation [29–33]. The veracity of indirect studies has been generally supported by direct measurements of NO and the enzymatic activity of the relevant NO synthase. Nevertheless, pharmacological studies are reliant upon the specificity of the NOS inhibitor or NO donor and should be interpreted with caution.

Nitric oxide is synthesized by a variety of cell populations in the pulmonary mucosa, submucosa, muscle, nerves and endothelium [32–38], reflecting the diverse functions NO assumes in the regulation of pulmonary physiology and inflammation. NO is generated catalytically by a family of three specialized nitric oxide synthases (NOS), which are similar to NADPH-cytochrome P-450 reductase [39, 40]. Each NOS isoform is unique but shares sequence homology [41], suggesting a common genetic ancestry, and all isoforms convert L-arginine to L-citrulline [42]. A byproduct of this reaction is the oxidation of a guanidino nitrogen group of L-arginine to the free radical nitric oxide, via an unstable reaction intermediate, N^G-hydroxy-L-arginine [3, 43, 44]. The NOS isoforms differ from one another with regard to cofactor requirements, tissue distribution, transcriptional regulation and post-translational modification [3, 45], but for convenience have been classified into two major types [43], constitutive and inducible, the latter requiring new protein synthesis in order to become functional [3]. There are two constitutive isoforms, termed ecNOS (endothelial NOS) and bNOS (brain NOS), and a single inducible isoform, iNOS (inducible NOS).

NO synthases constitutively expressed in the lung under physiological conditions serve an important homeostatic role in directing blood flow to

oxygenated alveoli [46], facilitating the transition to an extrauterine circulation [47, 48], maintaining pulmonary capillary pressure [46, 49], inhibiting neutrophil adhesion [21], and augmenting vascular patency via inhibition of platelet activation [50]. Although ecNOS and bNOS are expressed constitutively, the amount of NO synthesized by these enzymes is subject to allosteric regulation [8, 51]. The complexity of the NOS enzyme presents many targets for endogenous regulation, including the requirement for substrates (L-arginine [25] and oxygen [52]), cofactors (NADPH, FMN, FAD, tetrahydrobiopterin and calmodulin) [53] and the phosphorylation of critical amino acids [25, 53–55]. NO itself also potently inhibits the activity of NOS, exerting a negative feedback effect on NO synthesis [56]. In general, acute changes in the level of constitutive NO synthesis are regulated by the attachment and dissociation of the cofactor calmodulin to an allosteric regulatory site [57]. In the presence of calcium, calmodulin binds readily and permits electron flow from NADPH to the heme group of NOS [8]. When the level of cytosolic free calcium is lower, calmodulin dissociates from the enzyme and NO synthesis ceases [57]. By this means, fluctuations in intracellular calcium concentration effect rapid changes in NO synthesis [58]. Under physiological conditions, the level of intracellular calcium is itself acutely regulated by multiple agents, such as acetylcholine, bradykinin [59] and serotonin [59]. In the pulmonary circulation, for example, these vasoactive substances bind to receptors on the luminal surface of the vascular endothelium, induce the opening of calcium channels, and trigger vascular relaxation [3, 7, 32].

Under resting conditions, the presence of calcium-dependent constitutive ecNOS and bNOS, determined by immunoreactivity, Northern analysis and the conversion of L-arginine to L-citrulline, has been detected in rat lung homogenates and cultured transformed cells derived from a pulmonary lineage [60, 61]. Localization of these isoforms to specific pulmonary tissues has been accomplished by immunohistochemistry. Not surprisingly, ecNOS has been identified in the pulmonary vascular endothelium of small and medium-sized blood vessels [60]. Unexpectedly, both ecNOS and bNOS have been detected in alveolar and serosal epithelial cells [60, 61]. Expression of ecNOS has been detected in cultured human bronchiolar epithelium believed to be of Clara cell lineage, where it may serve to modulate ion flux and/or secretory function [62]. Neuronal NOS mRNA expression has been detected in SV40-transformed human bronchial epithelial cells [63].

An inducible isoform of NO synthase, iNOS, is generally absent under quiescent physiological conditions but is induced *de novo* in response to immune stimulation [64]. In contrast to the constitutive isoforms, iNOS binds calmodulin avidly at low intracellular calcium concentrations [65, 66]. Unrestrained synthesis of the inducible synthase might be expected to have deleterious consequences [11]. Not surprisingly, transcriptional regulation is under tight control by a variety of natural inhibitors, including inter-

leukin-10 (IL-10) [9], platelet derived growth factor [67], transforming growth factor-β (TGF-4β) [9] and corticosteroids [10]. Unlike the constitutive isoforms, which can synthesize relatively small amounts of NO rapidly for a short duration [11], the inducible isoform generates abundant NO for longer periods of time (several hours to days), at rates several orders of magnitude greater than that produced by the constitutive isoforms [68].

iNOS has been identified in alveolar macrophages from lung tissue obtained from clinical specimens of bronchiectasis and acute bronchopneumonia [69]. iNOS has also been characterized in animals stimulated by systemic or pulmonary inflammation in whole lung homogenates from the rat, [70], guinea pig (alveolar macrophages, interstitial macrophages) [14], and human bronchial epithelial cells [71, 72]. *In vitro* immune stimulation of pulmonary-derived cells induces iNOS formation and NO production, as shown in murine lung epithelial cells [24, 71, 72], rat pleural mesothelial cells [73], rat alveolar epithelial cells [27, 74], porcine pulmonary artery endothelial cells [75] and human bronchial and alveolar type 2 epithelial cells [63]. iNOS is also present in pulmonary vascular smooth muscle, as evidenced by the NO-dependent loss of vascular responsiveness of LPS-treated vascular rings [76] and vascular rings harvested from endotoxic rats [77]. These observations are supported by the finding of increased expression of iNOS mRNA in pulmonary arteries obtained from endotoxic rodents [77]. Interestingly, iNOS has also been identified in normal human airway epithelium *in vivo*, in the absence of apparent immunostimulation [78].

NO also may be produced by the lung from granulocytes and monocytes that infiltrate the mucosa and submucosa during exudative inflammation. Leukocytes typically exit the vascular compartment and penetrate the mucosa during sepsis or pneumonia in response to chemoattractant cytokines, such as interleukin-8 (IL-8). When activated by cytokines, leukocytes express high levels of iNOS [79] and may, thereby, contribute to the total NO release from the lung.

2. Physiological Regulation of Lung Function by Endogenous Nitric Oxide

2.1. Development Aspects

The developmental aspects of pulmonary physiology are of great importance, inasmuch as the transition to extrauterine life is dependent upon the adequate adaptation of the pulmonary circulation. Thus, it is not surprising that there are marked developmental changes in the NO-dependent pulmonary regulatory mechanisms during the late fetal and early postnatal period.

Basal pulmonary vasodilatory tone is modulated by NO during the late fetal period, as evidenced by the pulmonary vasoconstriction elicited by

intrapulmonary infusion of NOS inhibitors in fetal lambs [80]. NO may also be involved in the post-partum increase in pulmonary blood fow and decrease in pulmonary vascular resistance, since these alterations are reduced in the presence of NOS inhibitors.

Pulmonary NOS expression shows remarkable ontogenetic changes: in rats there is a more than threefold increase in ecNOS and neuronal NOS expression from the sixteenth to the twentieth day of gestation. The twentieth day also has the maximal expression: NOS expression shows a gradual decline in the postnatal period [81]. Similar observations were made when investigating a) endothelial NO production (assessed by measuring endothelium-dependent arterial cGMP production) in fetal and newborn lambs [62] and b) the vasoconstrictor effect of NOS inhibitors in isolated perfused lungs from one- and 7-day-old piglets [82]. The up-regulation of NOS may serve to increase the vasodilatory capacity of the lung during birth and in the early postnatal period.

2.2. Maintenance of Blood Flow

Agents that increase intracellular calcium by activating the influx of extracellular calcium or releasing calcium from intracellular stores, cause endothelium- and NO-dependent relaxation in most pulmonary vessels *in vivo* and *in vitro*. Such endothelium-dependent vasodilators include acetylcholine, ATP, ADP, substance P, bradykinin, serotonin and norepinephrine [83–85]. Important from a physiological point of view is the finding that pulsatile flow is a potent stimulant of endothelium-derived NO production. The mechanism of the vasodilatation is the NO-induced activation of guanylyl cyclase and consequently the decrease in intracellular calcium concentration in vascular smooth muscle cells [85, 86]. While in the systemic circulation veins seem to produce less NO than do arteries both basally and upon stimulation [83, 84, 87], in isolated pulmonary blood vessels, veins have higher basal NO output and NO induces more pronounced increases in the cGMP content of the underlying smooth muscle [32]. These latter observations in isolated vascular rings appear to be in contrast to data obtained in isolated perfused lungs where the increases in total pulmonary vascular resistance were the result of increases in the arterial component of vascular resistance [88]. More importantly, in this more physiological setting, the increases in total pulmonary vascular resistance in response to NOS inhibition increased with the increased flow rate, suggesting a tight coupling of basal release of NO to pulmonary blood flow [88].

Inhibition of the basal release of NO by NOS inhibitors results in a rapid, prolonged, and L-arginine-reversible vasoconstriction and an increase in the resistance of most vascular beds, including the pulmonary vascular bed of most species including humans [89–91]. In normal human volunteers,

inhibition of NOS with L-NMA (1 mg/kg/min) reduced basal NO production by 65% and increased pulmonary vascular resistance by approximately 40%, resulting in a 15% increase in mean arterial blood pressure but no alteration in mean pulmonary arterial pressure [91]. In contrast to certain other vascular beds, exercise does not appear to up-regulate NOS activity in the lung [92]. Basal NO production due to up-regulation or inhibition of ecNOS activity changes in a variety of pathophysiological conditions (see below).

It is important to point out that it is generally assumed that NO from the physiologically predominant isoform, ecNOS, is not dependent on extracellular substrate (see above). In contrast to these data, recent observations in humans show that oraly administered L-arginine increases exhaled NO in normal human volunteers [25]. The mechanism of this increase is unclear, but there are two possibilities which may explain these findings: 1) In contrast to the given earlier data, ecNOS in the pulmonary circulation is somewhat dependent on extracellular L-arginine; or 2) the increased NO production is due to increased NO production by iNOS, constitutively present in human pulmonary epithelial cells (see above).

2.3. Regulation of Platelet and White Cell Functions

Other important functions of endothelium-derived NO include the regulation of adhesion and activation of circulating blood cells. NO inhibits adhesion and activation of platelets [93, 94]. The inhibition by NO of the adhesion of platelets has been demonstrated in cultured pulmonary epithelial cells: the effect, similar to the vasodilatory effect of NO, is mediated by increases in intracellular cGMP levels in the target cells, in this case platelets [95]. In rabbits, inhibition of ecNOS has been shown to enhance the pulmonary accumulation of platelets in response to intravenous administration of adenosine diphosphate, platelet activating factor or thrombin [96]. In a more chronic setting, NO derived from the vascular endothelium may regulate the expression of endothelial adhesion molecules, such as P-selectin [97].

In addition to regulating platelet reactivity, NO from ecNOS regulates the adhesion of neutrophils [98–100]. Inhibition of endothelial NO production can cause microvascular leakage, implicating NO in the control of vascular permeability [98–100]. In rabbits, inhibition of ecNOS has been shown to enhance the pulmonary accumulation of neutrophils in response to intravenous administration of platelet activating factor [96]. Conversely, administration of NO to isolated lungs perfused with activated neutrophil granulocytes has been shown to reduce pulmonary injury [101].

2.4. Hypoxic Pulmonary Vasoconstriction

Hypoxic pulmonary vasoconstriction (HPV) is an essential physiological regulatory mechanism, which serves to match ventilation and perfusion to preserve arterial oxygenation. Impaired HPV response results in ventilation–perfusion mismatch and increased intrapulmonary shunt-flow. Despite intensive research in this area, the mechanism of HPV remains unclear. Initial *in vitro* studies have demonstrated that severe hypoxia impairs endothelium-dependent relaxations, and that the contractions that develop in hypoxic blood vessels are due to inhibition of basal NO formation [102]. Moreover, in spontaneously breathing rabbits, inhibition of NOS with L-NAME elicited a pronounced decrease in arterial PO_2 [103]. These observations may have suggested that NO might be a regulator of ventilation-perfusion matching at normoxic ventilation, whereas inhibition of basal NO production in hypoxia may mediate HPV.

However, subsequent *in vitro* work and more definitive *in vivo* studies now suggest that hypoxic inhibition of pulmonary NO production does not mediate acute HPV. In fact, inhibition of NOS during HPV caused a marked additional increase in the pulmonary arterial tone, suggesting that hypoxic vasoconstriction does not result from a reduction of the basal release of NO [104–107]. NOS inhibition causes a redistribution of blood flow away from the hypoxic alveoli, thus increasing systemic oxygen tension [105]. This effect is even more pronounced when NOS inhibitors are applied in combination with inhaled NO [46].

In a careful recent investigation of the role of NO in the HPV response in perfused rabbit lungs, the increase in pulmonary vascular tone induced by alveolar hypoxia was preceded by a rapid decrease in exhaled NO concentration. In contrast, perfusate NO accumulation was not altered. Thus, alveolar hypoxia, while modulating NO production in the alveoli, does not affect the sources of NO which are involved in the regulation of vascular tone (i.e. NOS in the vascular endothelial cells) [22].

3. Pathophysiological Alterations in the Nitric Oxide Homeostasis of the Lung

3.1. Nitric Oxide Underproduction (Endothelial Dysfunction)

3.1.1. Persistent pulmonary hypertension: Experimental and clinical data support the view that a suppression of basal NO production contributes to the pathophysiology of idiopathic persistent pulmonary hypertension of the newborn (PPHN). This conclusion is based on the following evidence: 1. chronic inhibition of NOS *in utero* causes persistent pulmonary hypertension after birth [18], 2. infants with persistent pulmonary hypertension have reduced plasma L-arginine levels [47] and decrease L-arginine util-

ization for whole body NO biosynthesis [108], 3. there is an impaired vasodilator response to the endothelium-dependent vasodilator calcium ionophore A23187 in pulmonary vessels from animals with experimental pulmonary hypertension [109], and finally 4. mean urinary concentrations of nitrite/nitrate were lower in patients with PPHN than in controls without pulmonary disease [36, 108]. Nonetheless, a definitive conclusion that NO deficiency is the fundamental cause of idiopathic pulmonary hypertension is premature. First, plasma L-arginine depletion may be a consequence of many factors, including iNOS induction. Second, the relaxation in experimental PPHN is also impaired in response to authentic NO, suggesting that the responsiveness of the smooth muscle to NO or cGMP is impaired [109]. In sum, existing data suggest that endogenous NO production is impaired in PPHN, and thus NO inhalation therapy can be considered a 'replacement therapy' in the treatment of this condition.

3.1.2. Hypoxic pulmonary hypertension: In parallel with the reevaluation of the role of NO in hypoxic pulmonary vasoconstriction, the initial enthusiasm regarding the role of NO in the mediation of chronic pulmonary hypertension has gradually diminished. Initial experimental data and studies performed in human tissues demonstrated impairment of NO synthesis and/or release in chronic hypoxic pulmonary hypertension, which had been proposed to be involved in the development of excessive pulmonary vasoconstriction [110, 111]. In chronically hypoxic rats, for example, inhibition of NOS has been shown to cause increased pulmonary hypertension [112].

However, in more recent investigations, NOS inhibition had no effect on baseline perfusion pressure in isolated salt-perfused lungs from either control or chronically hypoxic rats. Similarly, pulmonary vasodilatory responses to vasopressin or a calcium ionophre were unaffected by chronic hypoxic exposure [113]. In patients with primary pulmonary hypertension low exhaled NO concentrations can be observed, but this change is a reflection of the reduced blood capillary volume in these patients rather than a decreased basal production of NO [114]. Moreover, in a rat study, direct measurements have actually demonstrated an increase in ecNOS activity in response to chronic hypoxia [115]. On the other hand, a recent immunohistochemical study demonstrates markedly diminished ecNOS in the vascular endothelium of pulmonary arteries with severe histological abnormalities (plexiform lesions) in patients with pulmonary hypertension [116].

Thus, the role of altered endogenous NO production in chronic pulmonary hypertension appears to be controversial. The fact that endogenous production of NO in pulmonary hypertension is not necessarily suppressed, however, does not diminish the value of experimental and clinical observations that inhaled NO reduces the degree of pulmonary hypertension in these conditions (see below).

3.1.3. Ischemia-reperfusion injury and cardiopulmonary bypass: Impairment of the endothelium-dependent vasodilatory response has been reported in the pulmonary vasculature after cardiopulmonary bypass, in agreement with data from other types of ischemia-reperfusion injury. In an ovine model, total cardiopulmonary bypass of 90 minutes' duration, followed by 60 minutes of reperfusion, converted the endothelium-dependent response of pulmonary microvessels from vasodilatation to vasoconstriction [117]. A similar alteration has been observed clinically, in which there is a marked diminution of the pulmonary vasodilator response to acetylcholine after cardiopulmonary bypass [118]. The absence of local NO production at sites of endothelial injury may enhance platelet aggregation and stimulate the release of mediators, which under physiological conditions are vasodilators (e.g. ADP) but which in the setting of vascular injury have a vasoconstrictive action. Endothelial injury is also exacerbated by the increased neutrophil adhesion and activation that follow from diminished local concentrations of NO in the vicinity of the endothelium.

The mechanism of endothelial dysfunction in ischemia-reperfusion injury may be related to the formation of the toxic oxidant peroxynitrite during reperfusion (see also below). The production of peroxynitrite, and the consequent increase in lipid peroxidation, has been demonstrated recently in a perfused lung model [119]. In the early phase of reperfusion injury, inhaled NO actually enhances pulmonary injury due to increased production of peroxynitrite [21]. Peroxynitrite has the ability to initiate oxidant reactions and to cause endothelium-dependent relaxations [120]. Depending on the local ratio of NO and superoxide anion, inhaled NO can either increase peroxynitrite production, thereby worsening injury, or alternatively, reduce the oxidant reactivity of peroxynitrite (see below) and thus limit tissue injury.

In addition to these acute changes, expression of iNOS has been reported in human lung during the delayed stage of cardiopulmonary bypass [26]. Induction of iNOS and enhanced formation of NO may then further contribute to tissue injury (see below).

3.2. Nitric Oxide Overproduction (iNOS Induction)

3.2.1. Circulatory Shock: In rodent models of septic shock, convincing experimental evidence supports the view that expression of iNOS in macrophages, vascular smooth muscle cells, endothelial cells, epithelial cells and other cell types significantly contributes to the cardiovascular failure and multiple organ dysfunction syndrome [54, 121]. Similar to the alterations in the reactivity of peripheral arteries and veins during sepsis, NO-mediated hyporeactivity in response to vasoconstrictor agents and its restoration by inhibitors of iNOS, such as aminoguanidine, have been demonstrated in the pulmonary artery [122, 123]. It is generally believed

that induction of iNOS in response to LPS or microorganisms serves antimicrobial purposes (see below), but large quantities of NO or peroxynitrite cause damage to the host.

In rodent models of endotoxin shock, the most pronounced iNOS induction is observed in the lung [124]. Similar to endotoxic shock, induction of iNOS can be seen in the lung after several hours of severe hemorrhagic shock [125] and in response to gram-positive bacterial cell wall components [126]. Induction of iNOS in the lung of endotoxemic rats may be related to the induction of the enzyme in pulmonary endothelial cells, vascular smooth muscle cells and/or resident or adhering mononuclear cells [14, 37, 127, 128]. Together with the induction of iNOS, LPS also stimulates the induction of an L-arginine transporter, as demonstrated in pulmonary artery endothelial cells [129]. Increased transport of the substrate of NOS presumably contributes to the high output of NO under inflammatory conditions.

Several recent studies have confirmed the involvement of endogenous tumor necrosis factor-α (TNF-α), IL-1β and interferon-gamma (IFN-γ) in the induction of iNOS in various animal models of circulatory shock. In a rat model of shock caused by *Pseudomonas aeruginosa*, a monoclonal antibody to TNF-α markedly inhibited the increase in plasma nitrite/nitrate levels [130]. In peritoneal macrophages obtained from mice treated with an immunostimulant prepared from *Mycobacterium bovis*, a monoclonal antibody against TNF-α also suppressed nitrite production [295]. In the lung of rats challenged with LPS, both a monoclonal antibody to TNF-α and an antibody to IL-1β inhibited iNOS induction [37, 131, 132]. Similar to the *in vivo* situation, endotoxin-stimulated NO synthesis in cultured pulmonary artery endothelial cells is mediated through an autocrine pathway involving the endogenous production of TNF-α and IL-1β [75].

Induction of iNOS in the lung can be inhibited by glucocorticoids such as dexamethasone [133]. The pulmonary induction of iNOS is also regulated by *endogenous* glucocorticoids, as evidenced in adrenalectomized animals (in which the expression of iNOS by LPS is more pronounced) [133] and during endotoxin tolerance (where the induction of iNOS by LPS is suppressed due to up-regulation of circulating glucocorticoids) [134]. The inhibition of iNOS induction by glucocorticoids may be related to the steroid-induced expression of the endogenous anti-inflammatory peptide lipocortin-1 [135].

Whereas in the systemic circulation the role of iNOS in the derangement of cardiovascular function and organ failure is well defined, there is only limited evidence of the pathophysiological role of iNOS in the lung during shock. The earliest changes observed with septic shock-induced ARDS include extravasation of intravascular fluid, also known as pulmonary transvascular flux. In a recent study, selective inhibition of iNOS activity with aminoguanidine and S-methyl-isothiourea or inhibition of the expression of iNOS with dexamethasone inhibited the increase in transvascular

flux (as measured by the Evans Blue method) associated with LPS injection. These *in vivo* studies are in agreement with *in vitro* observations showing that immunostimulation induces a NO-mediated increase in permeability in pulmonary epithelial cell monolayers [136]. Thus, it appears that large amounts of NO generated by pulmonary iNOS during endotoxemia in rodents are deleterious to the lungs and increase epithelial permeability. Additional deleterious consequences of iNOS induction include suppression of sodium transport in pulmonary epithelial cells [136] and of surfactant synthesis in type II pneumocytes [137]. The latter effect appears to be mediated by cyclic GMP [137]. In addition, iNOS expression by TNF-α in tracheal epithelial cells has been proposed to cause mucin hypersecretion [4].

It is possible that the final cytotoxic mediator which mediates pulmonary injury is peroxynitrite, a potent oxidant produced from NO and superoxide radical (see below). Peroxynitrite in the lung may have additional toxic actions, including cellular energy depletion, glutathione depletion and surfactant damage (see below).

An interesting experimental therapeutic approach combines systemic NOS inhibition with NO inhalation therapy [138, 139], resulting in greater systemic oxygenation and increased survival compared to the two separate approaches. Since NO is known to inhibit the activity of iNOS directly [56], it would be possible for NO inhalation both to improve lung perfusion and inhibit iNOS activity directly, thereby reducing the endogenous production of NO. This, however, is not the case: NO inhalation (100 ppm for 48 h) does not appear to affect pulmonary iNOS activity *ex vivo* [140].

3.2.2. Other Proinflammatory Conditions: In addition to endotoxic and hemorrhagic shock, there are a number of other conditions associated with expression of iNOS in various cell types in the lung. As mentioned above, there is a postoperative increase in exhaled NO [141] and an induction of iNOS after cardiopulmonary bypass [26]. In addition, intestinal ischemia-reperfusion has been reported to induce pulmonary injury via the induction of iNOS in the lung [142].

Induction of iNOS in the lung in response to microorganisms is an important component of the host-defense system. For instance, *Legionella pneumophila* and *Cryptococcus neoformans* have been shown to induce iNOS in the lung, and inhibition of NOS with L-NMA resulted in enhanced growth or reduced clearance of these microorganisms in the lung several days after the initiation of the infection [34, 143]. Similarly, intratracheal injection of *M. tuberculosis* causes a rapid up-regulation of iNOS [144], which, according to *in vitro* data, may play a role in the overall host-defense response of the lung against *M. tuberculosis* infection. NO produced by iNOS may also play a role in the pathophysiology of viral pneumonia. In alveolar macrophages from rats infected with the Sendai virus, a major respiratory pathogen in rodents, increased cytoplasmic motility was

abolished by NOS inhibition. However, treatment of the animals with NOS inhibitors did not appear to affect the alterations in pulmonary morphology [145].

Induction of iNOS has also been demonstrated in pulmonary granulomata induced by intratracheal injection of dextran beads [146]. However, the role of iNOS in the resolution of the granulomata is unclear. Other pathophysiological conditions, in which the induction of iNOS has been demonstrated in the lung include hypercholesterolemia [147], asthma [13, 24, 70, 148, 149], cystic fibrosis [149], obliterative bronchiolitis [149] and lung injury in response to inhalation of pulmonary irritants [150]. Many of these observations are based upon biopsied tissue, which increases their clinical relevance. However, no data are yet available on the role of NO or iNOS (i.e. the effect of selective inhibition of iNOS) in these conditions.

4. Pharmacological Modulation of Pulmonary Function by Influencing Nitric Oxide Homeostasis

4.1. Nitric Oxide Inhalation Therapy

Acute pulmonary hypertension, resulting from a deficiency of NO production in the pulmonary vasculature [151] or from a non-NO related process, increases pulmonary capillary pressure, augments transvascular flux and increases right ventricular afterload [30]. Additionally, in the presence of right to left anatomic extrapulmonary shunts or intrapulmonary physiological shunts, pulmonary hypertension may contribute to systemic desaturation [152]. Chronic pulmonary hypertension may also induce remodeling of the pulmonary vasculature, with an irreversible smooth muscle hypertrophy [153]. An effective vasodilator, specific to the pulmonary circulation, is therefore desirable [154].

Recently, NO has been used to treat pulmonary hypertension, by delivery in its gaseous form to the lung during mechanical ventilation [154, 155]. Although the exact mechanism by which inhaled NO dilates the pulmonary vascular bed is unknown, it is presumed that NO is distributed to distal ventilated alveolar segments where it passes readily, due to its great lipophilicity, through the epithelium into the interstitial space. From there, NO passes through the vascular adventitia and reaches the cytosol of the arteriolar vascular smooth muscle [156] where it interacts with iron in the heme center of guanylyl cyclase. NO binding induces a conformational change in the enzyme that permits the catalysis of GTP to cGMP, with a subsequent alteration in intracellular calcium, and vascular smooth muscle relaxation. NO which instead passes through the vascular smooth muscle and endothelium into the vascular lumen is inactivated by its interaction with the

iron center in hemoglobin. In this manner, it is presumed that NO is a selective pulmonary vasodilator, since it immediately becomes ineffective in the systemic circulation [152]. Recent evidence suggests, however, that NO may circulate as a relatively stable adduct in the form of a nitrosylated hemoglobin species which could, in theory, cause systemic vasodilatation. In practive, however, inhaled NO does not appear to have any direct effect on systemic vascular resistance [154]. Although inhaled NO is thought to act primarily on pre-capillary vessels, there is evidence that it may also induce vasodilatation in the venous side of the pulmonary circulation, under conditions of extreme venoconstriction [156].

A variety of pediatric pulmonary pathologies are either associated with a deficiency of vascular NO production [157] or are thought to be potentially responsive to the selective pulmonary vasodilating properties of inhaled NO [30]. These include respiratory distress syndrome [158], lung hypoplasia (secondary to congenital diaphragmatic hernia [159]), primary pulmonary hypertension [160], ischemia-reperfusion injury (secondary to cardiopulmonary bypass [161]), hypoxemic respiratory failure (pneumonia [157] and ARDS [162]). Inhaled NO has also been considered as a bronchodilator, given its known action on the tone of nonvascular smooth muscle [163].

As mentioned above, extreme prematurity is associated in ovine models of respiratory distress syndrome with a relative deficiency of NO production and an elevated pulmonary vascular resistance [164]. As expected, inhaled NO has been shown to be an effective pulmonary vasodilator in these models; it improves systemic oxygenation, presumably by diverting ductal flow to the pulmonary circulation [158]. In term animals inhaled NO has been shown to be an effective pulmonary vasodilator in acquired pulmonary hypertension secondary to hypoxia with or without concomitant acidosis [152].

Under certain circumstances, inhaled NO is an effective pulmonary vasodilator in term animals with acquired pulmonary hypertension resulting from local and systemic inflammation. For example, inhaled NO has been shown to interfere with the recruitment of neutrophils to the pulmonary vasculature if administered four hours after ischemia-reperfusion injury [21]. Inhaled NO also effectively reduced pulmonary hypertension in a neonatal piglet group B streptococcal sepsis model [165] and in porcine models of endotoxic shock in which intravenous NOS inhibitors have been successfully used to correct refractory systemic hypotension [138, 139].

Therapeutic use of inhaled NO raises important issues of toxicity, alone and in combination with superoxide anion [71]. Indeed, NO is a poisonous gas and is rapidly fatal if inhaled in high concentration. Precise monitoring of inhaled NO concentrations is obligatory to assure delivery at the desired level. Nonetheless, even when delivered at concentrations of 100 ppm, NO may have toxic actions [71]. The basis for NO-mediated toxicity is

multifactorial and it is presently unclear which mechanisms are clinically relevant.

First, NO may interact with the iron center in hemoglobin, altering its oxidation state to form methemoglobin, a species unable to transport oxygen [157]. In the presence of adequate levels of erythrocytic methemoglobin reductase, the level of methemoglobin is usually less than several percent and is well tolerated [71] even after one week of continuous therapy [166].

Second, in the presence of high inspired concentrations of oxygen, NO in its gaseous form can react to produce nitrogen dioxide, an extremely toxic species even at levels less than 10 ppm [71]. This potential problem has been minimized by (1) mixing NO directly with oxygen before use, (2) monitoring nitrogen dioxide levels, and (3) including nitrogen dioxide scavengers in the ventilator circuit.

Third, NO may participate in deaminative reactions with DNA, potentially acting as a mutagen [71]. There have been no long-term follow-up *in vivo* studies of inhaled NO to exclude this possibility. Clearly, before inhaled NO can be considered as a chronic therapy, great attention would have to be given to issues of genetic injury.

Fourth, NO is a known inhibitor of platelet activation *via* its elevation of intracellular cGMP concentration [95]; thus, hemorrhage is a potentially important toxicity, particularly in premature infants who are at increased risk of cerebral bleeding. This issue remains to be explored.

Fifth, in combination with the superoxide anion, NO forms a potent oxidant, peroxynitrite, which mediates tissue injury during inflammation and ischemia-reperfusion [167]. Since inhaled NO is typically delivered in conjunction with high inspired levels of oxygen (which spontaneously generate superoxide anion [71, 167]), peroxynitrite formation is likely and potentially a very serious limitation on the clinical use of inhaled NO [71, 167]. Robbins *et al.* observed an increase in the minimal surface tension in pulmonary surfactant obtained from piglets treated for 48 hours with 90% oxygen and 100 ppm of NO, an admittedly high dose [71]. This combination also increased neutrophil chemotactic activity in piglet lungs [71], suggesting that the combination of inhalational NO therapy and hyperoxia might potentiale pulmonary inflammation. An additional point is that superoxide anion is generated in the lung during inflammatory conditions, such as pneumonia, asthma, sepsis, ischemia-reperfusion injury and ARDS. Eppinger *et al.* observed, for example, that inhaled NO was toxic if delivered coincidentally with an ischemia-reperfusion injury and that this injury was preventable by treatment with superoxide dismutase, an enzyme which scavenges superoxide anion [21]. The role of NO during reperfusion injury, however, is far from clear. Eppinger *et al.* have also observed that late treatment of ischemia-reperfusion injury with inhaled NO is salutary [21], and Poss *et al.* have noted that inhaled NO reverses the increase pulmonary vascular permeability induced in isolated rabbit lungs treated with hydrogen peroxide [168].

In light of the many potential toxicities of inhaled NO, several approaches are now under development to minimize NO delivery and peroxynitrite-mediated injury [169]. Ichinose *et al.* have observed that Zaprinast, an inhibitor of the phosphodiesterase that breaks down cGMP, prolongs the action of NO [169]. Recent reports indicating that very low doses of inhaled NO may be sufficient to induce physiologically meaningful pulmonary vasodilatation are encouraging and suggest that the therapeutic ratio of inhaled NO may be better than previously imagined [155, 161]. Adjunctive therapies that bolster endogenous antioxidant defenses in the lung may also have a role as prophylaxis for inhaled NO therapy. Until more is known about the relative importance of toxicity from inhaled NO, the current caution is justified [169] and the indiscriminate 'compassionate' use of this potentially noxious agent should be reconsidered.

The initial clinical trials of inhaled NO followed pioneering studies in sheep at Massachusetts General Hospital in the early 1990s [152]. Roberts *et al.* observed that inhaled NO, at doses up to 80 ppm, improved oxygenation in term infants with pulmonary hypertension from a variety of causes [160]. Discontinuation of the gas resulted, in general, in a loss of the vasodilating effect [160]. Lonnqvist *et al.* studied 14 neonates and children with pulmonary hypertension and found that most (10/14) could be classified as 'responders' to inhaled NO [157]. Variation in response in different children and in different clinical settings has been confirmed in multiple subsequent clinical studies. A variety of explanations have been advanced for the variation in clinical response to inhaled NO [170]. First, the underlying disease may not involve a reversible vasoconstrictive process affecting pulmonary vasculature (anatomic heart disease, fixes pulmonary vascular disease, hypoxia with normal pulmonary vascular resistance). Second, the dose of NO may be enough to ablate hypoxic pulmonary vasoconstriction, thereby worsening ventilation-perfusion mismatch.Third, NO toxicity may exacerbate the underlying pathology, perhaps masking any potential benefit derived from its effect on the pulmonary vascular tone.

In contrast to Roberts *et al.* [160], Kinsella *et al.* [171] noted that a rapid improvement in infants treated for pulmonary hypertension with inhaled NO was achievable at lower doses (20 ppm for four hours) and was sustained at very low doses (6 ppm). In agreement with these findins, Finer *et al.* noted that low-dose inhaled NO was useful in treating pulmonary hypertension and there seemed to be no advantage in using more than 5 ppm [172]. These promising initial clinical investigations led to the establishment of five multicenter randomized trials in the US and Canada, with the specific aim of evaluating the role of inhaled NO in the treatment of infants with pulmonary hypertension [173]. The early pilot studies also encouraged the proliferation of uncontrolled studies, isolated 'compassionate use' of inhaled NO, and unsupported claims suggesting that NO therapy should be combined with other equally controversial modes of ventilation, including

high frequency oscillatory ventilation and liquid ventilation [170]. This level of interest in NO therapy reflects both its promise and the inadequacy of conventional treatment. It should be borne in mind that not one controlled trial of inhalational NO therapy has yet shown an improvement in clinical outcome, a reduced need for ECMO or a decrease in the duration of mechanical ventilation or oxygen administration. Hopefully, the ongoing multicenter clinical trials will definitively establish the proper place for NO in the management of pulmonary hypertension, although there is now a real probability that these trials may never be completed [173].

Inhaled NO has also been tested clinically as a treatment for lung hypoplasia secondary to congenital diaphragmatic hernia. Karamanoukian *et al.* reported on a nonrandomized, multicenter trial of nine neonates treated with 80 ppm of inhaled NO [159]. The authors concluded that NO was only effective after treatment with ECMO [159]. Kinsella *et al.* reported a salutary effect of NO in this condition, with eight out of nine infants improving but only in combination with high frequency ventilation [171]. The authors suggested that this type of ventilation was necessary to achieve the level of lung inflation required for adequate NO diffusion into the pulmonary vasculature [171].

Inhaled NO has been suggested as a novel treatment for bronchial asthma [163] on the basis that NO inhibits smooth muscle contraction. Clinical studies by Pfeffer *et al.* have not confirmed any benefit in the pediatric age group [163]. Recent data indicating that asthmatics have increased levels of exhaled NO suggest that reactive airway disease may be an NO-mediated inflammatory condition [163]. Asthma characteristically responds favorably to glucocorticoids, which are potent inhibitors of the inducible NOS in most systems [64]. Thus, the use of inhaled NO in asthma is probably of no benefit and may be deleterious. Treatment of asthma with isoform-selective inhibitors of the inducible NOS is very promising and is the subject of ongoing studies.

Inhaled NO may be useful in the treatment of pulmonary vascular hypertension following cardiopulmonary bypass [31, 161, 164, 166, 174, 175]. In pilot studies, postoperative use of inhaled NO has resulted in a decrease in pulmonary vascular resistance and an increase in oxygenation [166] without a concomitant decrease in arterial pressure [31, 161, 164, 166, 174, 175]. Nonresponders are typically those patients with normal pulmonary vascular resistance [164]. Unfortunately, there have been no prospective, randomized, blinded trials of inhaled NO in the postoperative management of congenital heart disease.

4.2. Selective Inhibition of Nitric Oxide Synthase Isoforms

Since the three NOS isoforms serve various physiological and pathophysiological roles, it is important to target them selectively for therapeutic

purposes [176]. In shock and inflammation, NO derived from iNOS is responsible for most of the pathophysiological actions, whereas, in many cases, ecNOS has beneficial and protective roles and so its inhibition is not advantageous.

Both ecNOS and iNOS can be inhibited (to a variable degree) with N^G-substituted L-arginine analogues, e.g. N^G-methyl-L-arginine (L-NMA). Some analogues of L-arginine do exhibit some isoform selectivity, mostly towards the constitutive isoforms. N^G-cyclopropyl-L-arginine shows a preference for bNOS over iNOS *in vitro*, while N^G-nitro-L-arginine (L-NA) and N^G-nitro-L-arginine methyl ester (L-NAME) show selectivity toward ecNOS. N^G-methyl-L-arginine (L-NMA) and N^G-amino-L-arginine show no marked preference for either isoform [176]. Despite their lack of marked isoform selectivity and certain nonspecific effects (see below), L-arginine-based inhibitors have been used widely in the last five years to elucidate the pathophysiological importance of NO and have demonstrated beneficial effects in various forms of shock and inflammation.

Many recent experimental efforts have focused on the development of iNOS-selective inhibitors. Among the aminoacid based inhibitors, L-N^6-(1-iminoethyl)lysine has been shown to exhibit selectivity for iNOS and has potent anti-inflammatory effects [177, 178]. Of the non-arginine-based small molecules, guanidines [179, 180], S-alkylisothioureas [181–183], amidines [184] and mercapto-alkylguanidines [185] have been reported to inhibit NOS potently, some of them with selectivity for iNOS.

Aminoguanidine is a mechanism-based, irreversible inactivator of iNOS [186]. Aminoguanidine has beneficial effects in various experimental models of inflammation and shock, although the potency of aminoguanidine *in vivo* is rather low [187–190]. It restores contractile responses in pulmonary artery of animals treated with endotoxin [122]. The most potent guanidino inhibitors of iNOS reported are mercaptoalkyl-guanidines, in particular mercaptoethyl- and mercaptopropyl-guanidines, or certain dimerization products, such as guanidinoethyldisulfide [185]. These compounds are far more effective than L-NMA at inhibiting iNOS in whole cells, but only limited data are so far available on their biological activity *in vivo*. S-methyl-isothiourea reduces the endotoxin-induced increase in pulmonary leakage in endotoxin-treated rats [191]. Based on the available data, it appears likely that selective inhibitors of iNOS, alone or in combination with NO inhalation therapy [138, 139], will play a significant role in the experimental therapy of inflammatory pulmonary diseases.

5. Interaction of Nitric Oxide with Other Mediators of Inflammation in the Lung

5.1. Nitric Oxide and Oxygen-Centered Free Radicals

Simultaneous generation of nitric oxide and superoxide favors the production of the toxic reaction product, peroxynitrite anion ($ONOO^-$) [192, 193]. In *in vitro* systems, the ratio of superoxide and NO determines the reactivity of peroxynitrite: excess NO reduces the oxidation elicited by peroxynitrite [120, 194, 195]. There is evidence that peroxynitrite is formed *in vivo*, as the end-product of specific oxidative processes triggered by peroxynitrite can be detected (see below). The oxidant reactivity of peroxynitrite is mediated by an intermediate with a biological activity of hydroxyl radical, which is not hydroxyl radical *per se*, but rather peroxynitrous acid or its activated isomer [194].

Immunohistochemical evidence demonstrates that peroxynitrite is produced in the lung in ARDS, hyperoxic lung injury [196, 197], endotoxic shock [14] and ischemia-reperfusion injury [198]. Although there is no available agent to scavenge or neutralize peroxynitrite specifically, indirect evidence suggests that much of the toxicity of NO is in fact mediated by peroxynitrite. Peroxynitrite is more cytotoxic than NO or superoxide in a variety of experimental systems [199–204]. For instance, *in vitro* studies demonstrate that NO itself has extremely limited effects on aconitase activity, whereas peroxynitrite is an extremely potent inhibitor of aconitase under the same experimental conditions [199, 200]. Similarly, peroxynitrite, and not NO, is a potent initiator of DNA strand breakage [205]. Moreover, while NO inhalation therapy at lower concentrations does not appear to cause direct cytotoxicity, NO inhalation under conditions of oxidant stress (such as hyperoxia) enhances cytotoxicity via the generation of peroxynitrite [71]. Scavenging oxygen radicals or peroxynitrite limits the toxicity of NO-generating drugs [206, 207], suggesting that when large amounts of NO are generated in biological systems, there are sufficient amounts of basal superoxide (produced by the mitochondria, for example) to form peroxynitrite and cause consequent cytotoxicity. Additionally, when large amounts of superoxide are generated in the lung, such as during ischemia/reperfusion injury [21] or paraquat intoxication [12], peroxynitrite formation from superoxide and NO (generated by ecNOS) will mediate pulmonary injury.

What are the major components of pulmonary injury elicited by peroxynitrite? Peroxynitrite is higly reactive and oxidizes sulfhydryl groups and thioethers; it also nitrosates hydroxylated aromatic compounds such as tyrosine [208]. Tyrosine nitrosation may lead to dysfunction of proteins, including superoxide dismutase [209], cytoskeletal actin [157] and neuronal tyrosine hydroxylase [198]. Peroxynitrite directly inactivates certain proteins and enzymes that are important for the energetic balance of the

cells. For instance, peroxynitrite is an extremely potent inhibitor of mitochondrial and cytosolic aconitase [199, 200]. There is also evidence that peroxynitrite can cause covalent modification of an active thiol site of glyceraldehyde 3-phosphate dehydrogenase [210], disruption of the zinc-thiolate center at the active site of enzymes [211], inhibition of membrane Na^+/K^+ ATP-ase activity [212] and inactivation of membrane sodium channels [213]. Exposure of peroxynitrite to isolated mitochondria has potent direct inhibitory effects on the mitochondrial respiratory chain [199, 203, 214, 215]. Addition of peroxynitrite to pulmonary type II cells, macrophages and vascular smooth muscle inhibits membrane Na^+/K^+ ATP-ase activity, sodium uptake and mitochondrial respiration [205, 212].

In addition, formation of peroxynitrite in pulmonary epithelial cells can lead to depletion of the endogenous glutathione pools [216]. It is conceivable that overproduction of peroxynitrite in the lung, by inhibiting energy-generating processes, would induce a profound deterioration of the energetic status of the affected cells.

Peroxynitrite induces direct damage of pulmonary surfactant proteins [197, 217, 218]. Although there are no *in vivo* data available, it is conceivable that peroxynitrite-induced damage to the surfactant system may compromise pulmonary function. Additional toxic effects of peroxynitrite may include its effects on lipids with the triggering of lipid peroxidation, resulting in malondialdehyde and conjugated diene formation [219] and the formation of nitrito-, nitro-, nitrosoperoxo- and/or nitrated lipid oxidation adducts [215].

Another important interaction of peroxynitrite occurs with nucleic acids, with the production of 8-hydroxydeoxyguanosine [220] or 8-nitroguanine [221]. Peroxynitrite can cause DNA cleavage in solutions of end-labeled DNA restriction fragments [222] and can initiate DNA nicking in the supercoiled plasmid pBR322 [223]. DNA single strand breakage, initiated by endogenous or exogenous peroxynitrite, is a potent trigger of poly-ADP ribosyl synthetase (PARS) activation [224–226]. PARS is a proteinmodifying and nucleotide polymerizing nuclear enzyme [227]. Activation of PARS results in the cleavage of NAD^+ into ADP-ribose and nicotinamide. PARS covalently attaches ADP-ribose to various nuclear proteins, such as histones and PARS itself. Activation of PARS can rapidly deplete NAD^+, slowing the rate of glycolysis, electron transport and ATP formation, resulting in cell dysfunction and cell death [225, 226, 228].

The reactivity and decomposition pathways of peroxynitrite are strongly influenced by the chemical environment. In the presence of plasma, proteins, glucose or glutathione, peroxynitrite can form intermediates which act as NO donors [229, 230]. This is particularly interesting in the light of the finding that NO inhibits the oxidant activity of peroxynitrite (see above). In solutions containing carbonate, peroxynitrite forms an adduct with carbonate, which then may decompose to yield the toxic HCO_3^- radical [231]. In plasma, peroxynitrite oxidizes ascorbic acid, uric acid, tyrosine,

and -SH groups of plasma proteins [232]. Scavengers of peroxynitrite include uric acid, cysteine, glutathione, ascorbic acid, desferrioxamine, vitamin E and Trolox, its water-soluble analogue and synthetic manganese-mesoporphyrins [233]. There may be a delicate balance between peroxynitrite-mediated oxidant processes and endogenous antioxidant pathways that limit the reactivity of peroxynitrite [234].

5.2. Nitric Oxide and Cyclooxygenase Metabolites

NO can activate or inactivate a variety of enzymes in a cGMP-independent fashion. NO-mediated activation of cyclooxygenase (COX) has been described in various cells including macrophages [235–237], and is related to the reaction of NO with the iron-heme center in the active site of the enzyme. On the other hand, NO at high concentrations inhibits COX activity [235]. Activation of COX by NO may have proinflammatory effects in the lung.

Cyclooxygenases are similar to nitric oxide synthases in the sense that they exist in two distinct isoforms, a constitutive and a cytokine-inducible. The expression of the inducible isoform (COX-2) has been demonstrated in the lung, in response to proinflammatory stimuli [238, 239]. It is therefore conceivable that large amounts of NO, produced by iNOS, activate COX-2 during pulmonary inflammation, and inhibition of NOS may reduce the production of NO as well as the production of prostaglandins [240, 241].

6. Future Trends

In relation to NO overproduction and NO toxicity, a radical shift in thinking has emerged in the last two years, suggesting that peroxynitrite, and not NO *per se*, is the relevant species in pulmonary free radical-mediated injury. The implications of this new paradigm are relevant to the clinical manipulation of NO-related diseases. Agents that nonspecifically ablate all NO production may reduce peroxynitrite formation but at an unacceptable cost of pulmonary vascular hypertension and right ventricular strain. Isoform-selective inhibition of the inducible NOS isoform may preserve the beneficial action of the constitutive low levels of NO production but will fail to eradicate peroxynitrite formation resulting from the reaction of superoxide with the remaining NO produced by ecNOS. Thus, the efficacy of NOS inhibitor therapy as a single isolated approach to the management of pulmonary inflammation is in question. What is probably required is a combination of those agents which 1) *diminish* peroxynitrite formation via the selective inhibition of iNOS, 2) *spare* ecNOS activity in order to avoid unwanted effects of tissue ischemia, platelet activation and neutrophil adhesion, and 3) *scavenge* residual peroxynitrite.

In relation to NO inhalation, there are many experimental approaches aimed at minimizing the toxic effects of NO and localizing the effects of NO to the pulmonary bed. These approaches, coupled with the application of NO inhalation therapy to more tightly defined groups of patients, should help to improve the clinical efficacy of NO inhalation therapy.

7. Conclusions

NO has an established role as a regulator of pulmonary physiology during fetal development and childhood. Alone and in combination with oxygen-centered free radicals, NO also represents an important pathophysiological mediator, contributing to inflammation in a wide range of acquired pulmonary diseases. Until recently, the interest in the biology of NO has been confined to the realm of basic investigation. With the advent of novel delivery systems to provide exogenous NO and the discovery of isoform-selective NOS inhibitors to reduce NO formation, there are now new therapeutic means to modulate pulmonary vascular resistance and parenchymal inflammation.

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