

# Inhaled nitric oxide in 2003: a review of its mechanisms of action

*[L'inhalation de monoxyde d'azote en 2003 : une revue de ses mécanismes et de son action]*

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**Purpose:** To review the pulmonary and systemic effects of endogenous nitric oxide and inhaled nitric oxide administered to patients.

**Source:** A systematic search for experimental data, human case reports, and randomized clinical trials since 1980, the year of discovery of endothelium-derived relaxing factor.

**Principal findings:** Nitric oxide has pulmonary and systemic effects. Inhaled nitric oxide not only causes selective pulmonary vasodilation but also results in pulmonary vasoconstriction of the vessels perfusing non-ventilated alveolae. The systemic effects of inhaled nitric oxide, which include modulation of the distribution of systemic blood flow, increase in renal output, interaction with coagulation, fibrinolysis and platelet functions, alteration of the inflammatory response, are described and the mechanisms of nitric oxide transport are explained. The possible toxicity of inhaled nitric oxide is also discussed.

**Conclusion:** The multiple effects of inhaled nitric oxide support its role as a pulmonary and extra-pulmonary medication.

**Objectif :** Revoir les effets pulmonaires et généraux de l'oxyde nitrique (NO) endogène et du NO inhalé, administré aux patients.

**Source :** Une recherche systématique de données expérimentales, d'études de cas humains et d'essais cliniques randomisés réalisés depuis 1980, année de la découverte de ce facteur relaxant d'origine endothéliale, dénomination qui lui fut attribuée à l'époque.

**Constatations principales :** Le monoxyde d'azote a des effets pulmonaires et généraux. Le monoxyde d'azote inhalé ne cause pas seulement une vasodilatation pulmonaire sélective, mais il provoque aussi une vasoconstriction pulmonaire des vaisseaux perfusant les alvéoles non ventilées. Les effets généraux de monoxyde d'azote, y compris la modulation de la distribution du débit sanguin, l'augmentation du débit rénal, l'interaction avec la coagulation, la capacité fonctionnelle de la fibrinolyse et des plaquettes, la modification de la réponse inflammatoire, sont décrits et les mécanismes du transport de

monoxyde d'azote sont expliqués. On discute également de la toxicité de monoxyde d'azote inhalé.

**Conclusion :** Les multiples effets de l'oxyde nitrique inhalé expliquent l'intérêt qu'il présente comme médication pulmonaire et extrapulmonaire.

**I**N 1980, Furchgott and Zawadzki<sup>1</sup> discovered that endothelial cells stimulated by acetylcholine released a vasodilator. Initially named endothelium-derived relaxing factor, its real nature was established several years later, and the molecule was identified as nitric oxide (NO).<sup>2,3</sup> Over the past two decades, many of NO's biological mechanisms and therapeutic indications have been elucidated. This review summarizes the biological effects of NO.

## Synthesis of NO

### Endogenous NO

Endogenous NO is synthesized by NO synthase (NOS), which combines O<sub>2</sub> with L-arginine to produce NO and L-citrulline.<sup>4,5</sup> The reaction requires nicotinamide adenine dinucleotide phosphate, flavin adenine dinucleotide, flavin mononucleotide, and tetrahydrobiopterin as cofactors.<sup>6,7</sup> Three types of NOS have been cloned: type I (neuronal or nNOS), type II (inducible or iNOS), and type III (endothelial or eNOS). Neuronal NOS and eNOS are constitutive calcium calmodulin-dependent enzymes and produce NO in nanomolar concentrations when activated by different agonists that increase intracellular calcium. Two isoforms of nNOS have been documented by antisense mapping.<sup>8</sup> Inducible NOS is synthesized in

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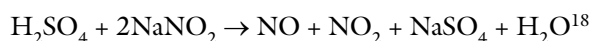
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response to several inflammatory mediators and produces NO in micromolar concentrations in a calcium-independent manner.<sup>9,10</sup>

*In vivo* synthesis of NO occurs in the lungs in the vascular endothelium, epithelial cells,<sup>11</sup> nerve cells,<sup>12</sup> smooth muscle cells, and inflammatory cells such as macrophages. In addition, the cells of the upper airways, especially in the nose and paranasal sinuses,<sup>13,14-17</sup> generate large amounts of NO, which is inhaled whenever the patient inspires through the nose.

#### *Commercial production of NO*

*In vitro*, NO gas can be produced from the reaction of liquid sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) and liquid sodium nitrite (NaNO<sub>2</sub>), and then purified of nitrogen (N<sub>2</sub>), nitrous oxide (N<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>):



NO gas can be added to the inspiratory gas flow during the inspiratory phase and delivered to the patient at a precise concentration. Several companies have developed very efficient NO delivery systems that match NO administration with inspiratory gas flow.

NO is available in industrial and medical grades. Medical grade NO is higher in purity but is more expensive than industrial grade NO. Currently, most Canadian hospitals use industrial NO from different suppliers; however, at some point, the therapeutic standard will require medical grade NO gas. Unfortunately, medical grade NO is only available through the Special Access Program and has not yet received definitive approval from Health Canada.

In Canada, medical grade NO is available as ViaNOx™-H (Summit Technology Inc.) in C-size cylinders (2.18 L of 800 ppm NO) at \$2,728 Canadian. In the United States and Europe, INO Therapeutics sells a complete package that comprises a delivery apparatus, medical grade NO, staff training, and paramedical and technical support. The company charges for NO treatment on an hourly basis (US \$150·hr<sup>-1</sup> or Euro 150·hr<sup>-1</sup> for a maximum of 96 hr).<sup>19</sup>

#### **Biological mechanisms of NO**

NO stimulates soluble guanylate cyclase (sGC) and increases cyclic guanylate monophosphate (cGMP). The latter activates cGMP-dependent protein kinases that are abundant in the cerebellum, smooth and cardiac myocytes, platelets, and leukocytes.<sup>20</sup> In turn, the kinases mediate the cGMP-induced decrease in intracellular calcium concentration in vascular smooth muscles and produce relaxation and vasodilatation.<sup>21</sup>

#### *Pulmonary effects of NO*

NO has several potentially beneficial effects on pulmonary function by maintaining low pulmonary arterial pressures and sustaining normal vascular permeability. In addition to its vasodilatory effects, recent work in pigs suggests that *in vivo* NO production is inhibited by a blood-borne inhibitor of NOS (such as asymmetric dextromethylarginine or by direct inhibition of eNOS)<sup>22</sup> in the presence of inhaled NO (iNO). The effect is greater in hypoxic regions. Thus, iNO may have a dual effect: pulmonary vasodilatation in ventilated regions and pulmonary vasoconstriction in poorly ventilated or hypoxic regions. The overall effect is a decrease in pulmonary arterial pressures, attenuation of ventilation-perfusion mismatch, and improvement in oxygenation. Animal studies show that iNO is particularly effective in reversing hypoxia-induced pulmonary hypertension but is less potent in vasoconstrictor-induced pulmonary hypertension from thromboxane A<sub>2</sub> or protamine-heparin reaction.<sup>23,24</sup>

In addition to its effect on the pulmonary vasculature, NO has some antibacterial actions provided through formation of reactive nitrogen oxides like peroxynitrite.<sup>25</sup> NO also modulates ciliary beat frequency<sup>26</sup> and can inhibit or stimulate mucus secretion.<sup>27,28</sup>

Inhaled NO has been considered for a long time to be a selective pulmonary vasodilator that has no clinically significant effect on blood pressure and cardiac output. Its selective action results from the fixation of iNO to the heme moiety of the hemoglobin molecule after passing through the pulmonary vessel wall. NO is then oxidized to NO<sub>2</sub> and NO<sub>3</sub>. Hemoglobin is transformed to methemoglobin, which is secondarily reduced to hemoglobin by methemoglobin reductase.<sup>29,30</sup> Although iNO has no systemic hemodynamic effects, it does have extra-pulmonary activity.

#### *Effects of NO on coagulation*

NO interferes with platelet and leukocyte functions,<sup>31</sup> fibrinolysis,<sup>32</sup> restenosis,<sup>33</sup> and reperfusion injury<sup>34</sup> by inhibiting expression of adhesion molecules at leukocyte surfaces and by activating sGC, which lead to rapid increase in platelet cGMP and inhibition of platelet aggregation. NO also inhibits vascular smooth muscle cell proliferation, leading to decreased neointimal hyperplasia.<sup>35</sup>

#### *Effects on inflammation*

The mechanisms of action of NO on the inflammatory process are complex. Conflicting data on the effect of iNO on the pulmonary inflammatory response have been published, as both increases and decreases in inflammatory mediators have been observed in

patients suffering from severe acute respiratory distress syndrome treated by iNO.<sup>36-40</sup> The different effects depend on the concentration of NO, the local oxidation-reduction potential, and the presence of other inflammatory mediators and oxygen-derived free radicals.<sup>41,42</sup> Low amounts of NO derived from eNOS and nNOS are believed to be beneficial, whereas large quantities produced by iNOS contribute to the injury observed in different experimental models of inflammation. However, inhibition of iNOS may exacerbate injury in certain situations, suggesting that iNOS-derived NO may be protective as well.

NO interferes with nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B) and inhibits tumour necrosis factor (TNF) alpha activation of NF- $\kappa$ B through stabilization of I $\kappa$ B- $\alpha$  and increased I $\kappa$ B- $\alpha$  gene expression. NO modulates leukocyte-endothelial interactions and the infiltration of activated leukocytes into sites of inflammation by inhibiting the expression of adhesion molecules.<sup>43-47</sup> NO can have pro-inflammatory effects by increasing the expression of inflammatory proteins such as TNF- $\alpha$  and cyclooxygenase-2.<sup>48,49</sup>

Inhaled NO does not decrease the inflammatory response when inflammation is already in process and may increase peroxynitrite production, but its use very early or before the onset of inflammation may attenuate pulmonary and extra-pulmonary inflammatory processes. In acute cardiopulmonary bypass-induced pulmonary inflammation in pigs, iNO modulates the pulmonary inflammatory reaction by preventing pulmonary vasoactive responses and by preventing the production of inflammatory intermediates, such as matrix metalloproteinases.<sup>50,51</sup> Inhaled NO, given early but not late in the disease process, can prevent pulmonary endothelial dysfunction that follows endotoxin injection.<sup>52</sup> Inhaled NO reduces myocardial dysfunction in endotoxemic rats<sup>53</sup> and can prevent pulmonary hypertension after congenital heart defect repair in humans.<sup>54</sup> If these observations are confirmed by clinical trials, prophylactic iNO at non-toxic concentrations could be an excellent medication to reduce excessive inflammatory responses that follow medical or surgical pathologies.

#### *Systemic vascular effects of NO*

At the peripheral level, NO and O<sub>2</sub> are released as hemoglobin changes its configuration from the relaxed, (high NO and high O<sub>2</sub> content) to the tense (low NO and low O<sub>2</sub> content) form. NO is transported from the hemoglobin molecule through the cell membrane via anion exchange protein.<sup>55,56</sup> A number of intermediates and mechanisms have been described to explain the transfer of NO fixed to proteins to

cytosolic sGC, the NO receptor.<sup>57</sup> Plasmatic nitrite can also be reduced to NO in an acidic milieu.<sup>58</sup> Although iNO has no clinically significant global effect on systemic hemodynamics, it has a differential action on the circulation, decreasing blood flow if the NO sGC pathway is normal, and increasing it if NO sGC is inhibited, as can happen in arteriosclerosis.<sup>59</sup> It can also decrease endothelium-dependent relaxation in the rat aorta (personal communication; Éric Troncy) and in the pulmonary circulation.

#### *Other effects of NO*

Inhaled NO increases urine output without changing systemic hemodynamic parameters in pigs,<sup>60</sup> rats, and humans.<sup>61</sup>

#### **Adverse effects of NO**

##### *Toxicity*

Toxicity from iNO results from the formation of methemoglobin, NO<sub>2</sub>, and peroxynitrite.<sup>62</sup>

- Hb (Fe<sup>2+</sup>) + NO → MetHb (Fe<sup>3+</sup>) + NO<sub>3</sub>
- 2NO + O<sub>2</sub> → 2NO<sub>2</sub>
- NO + O<sub>2</sub> → ONOO<sup>-</sup>

Methemoglobinemia is generally uncommon as methemoglobin is catalyzed by red cell methemoglobin reductase, assuming the iNO concentration, pulse oximetry and clinical signs (e.g., cyanosis, dyspnea) are effectively monitored and the NO concentration is maintained within 80 ppm. Young children deficient in methemoglobin reductase may be at increased risk.

NO can be oxidized to NO<sub>2</sub>, a potent oxidant.<sup>63,64</sup> Concomitant administration of high iNO concentration (> 100 ppm) and high FiO<sub>2</sub> will accelerate this reaction. The upper level of acceptable exposure iNO<sub>2</sub> in humans is 2 ppm. The estimated one hour NO<sub>2</sub> dose resulting in 50% mortality (LD<sub>50</sub>) among humans is 174 ppm, and short-term exposure > 150 ppm NO<sub>2</sub> is generally fatal.

NO reacts with superoxide anion (O<sub>2</sub><sup>-</sup>) to produce peroxynitrite (ONOO<sup>-</sup>), a potent oxidant and proinflammatory mediator. Endogenous peroxynitrite plays an important role in the killing of microorganisms and tumour cells; however, excess exogenous or endogenous peroxynitrite in the lungs has potential adverse pulmonary effects, including surfactant alteration, macromolecular structural changes, metabolic injury, and induction of apoptosis.<sup>39,40,65</sup>

Peroxyntirite is a pulmonary irritant and an irreversible inhibitor of mitochondrial function. It destroys surfactant by lipid peroxidation, prevents further surfactant production by mitochondrial poisoning of alveolar type II cells,<sup>66</sup> and may consequently

enhance the tendency to alveolar collapse. It may also damage the pulmonary endothelium<sup>67,68</sup> and profoundly affect the production of endothelium-derived relaxation factor, leading to pulmonary hypertension and trapping of circulating cells in the pulmonary circulation.<sup>69</sup> Peroxynitrite can induce DNA breakdown and secondary polyadenyl ribose synthetase activity in order to repair DNA, leading to deficits in energy metabolism. Inhibition of polyadenyl ribose synthetase has a beneficial effect in different inflammatory syndromes such as reperfusion injury, sepsis, and diabetes;<sup>70,71</sup> activation of polyadenyl ribose synthetase consumes large amounts of adenosine diphosphate and may lead to deficits in energy metabolism.

#### *Rebound pulmonary hypertension and hypoxemia*

Life-threatening increases in pulmonary vascular resistance have been noted on acute withdrawal of iNO. Most studies have demonstrated that eNOS levels in the pulmonary vascular endothelium remain unchanged during iNO withdrawal, suggesting that inactivation or down-regulation of eNOS is one of the mechanisms of rebound pulmonary hypertension.<sup>72,73</sup> Inhaled NO also increases plasma endothelin-1 concentrations without enhancing gene expression in lamb. Endothelin-1 receptor blockade prevents the increase in pulmonary vascular resistance after NO withdrawal, indicating a role for endothelin-1 in rebound pulmonary hypertension.<sup>74</sup> In lamb, reduced NOS activity, associated with iNO therapy, may involve endothelin-1 receptor-mediated O<sub>2</sub><sup>-</sup> production.<sup>75</sup> NO may stimulate xanthine oxidase and cause an increase in cellular O<sub>2</sub><sup>-</sup> generation. A reaction between NO and O<sub>2</sub><sup>-</sup> would produce peroxynitrite, which could then inactivate eNOS protein.<sup>72,74,76</sup> Endothelin-1 receptor antagonists may prevent rebound pulmonary hypertension by protecting endogenous eNOS activity during iNO therapy.<sup>74</sup>

To prevent rebound pulmonary hypertension, iNO should be tapered off progressively without any attempt to terminate it if FiO<sub>2</sub> is higher than 50%. A decrease in oxygenation induced by NO withdrawal may be compensated by an increase in FiO<sub>2</sub>. In our clinical trial, we determined the daily optimal concentration of iNO (the lowest concentration with the greatest effect on oxygenation) and found that, as patients improved, the required iNO concentrations decreased.<sup>77</sup> Weaning from iNO was undertaken when optimal iNO concentration was 0.5 ppm and was successful in each patient. If weaning from iNO induces a large increase in pulmonary pressure with a decrease in oxygenation, it should be restarted and more progressive weaning should be undertaken. Drugs that poten-

tiate or increase the production of endogenous NO could be useful in difficult cases.

#### **Alternatives to inhaled NO**

Pulmonary vasodilatation can be achieved with soluble NO donors, phosphodiesterase inhibitors, prostaglandins, and endothelin receptor antagonists. NO nucleophile adducts, and NONOate (2-(dimethylamino) ethylputrescine-NO) have been used in animal models of pulmonary hypertension<sup>78,79</sup> and compared to inhaled sodium nitroprusside<sup>80,81</sup> and inhaled nitroglycerine.<sup>82,83</sup> The duration of action of these medications is longer than iNO and their pulmonary selectivity has been questioned, particularly for the NONOates. Currently, only inhaled sodium nitroprusside has been used in humans.<sup>84</sup>

The effect of iNO can be enhanced by phosphodiesterase V inhibitors (dipyridamol, zaprinast, sildenafil), which block cGMP degradation.<sup>85,86</sup> These medications can be inhaled and produce vasodilatation by enhancing the effect of endogenous NO; this is particularly true for sildenafil, which is not only a phosphodiesterase V inhibitor but also induces smooth muscle cells hyperpolarization through its action on potassium channels. Sildenafil could become one of the first treatments in pulmonary hypertension.<sup>87</sup> Milrinone and amrinone, phosphodiesterase III inhibitors that specifically metabolize cyclic adenosine monophosphate (cAMP), may potentiate iNO's effect in an additive fashion as they act on different pathways. Milrinone has been used in inhalation therapy in humans.<sup>88</sup>

Prostaglandin E1 and I2 increase cAMP and reduce pulmonary hypertension; *iv* prostacyclin (prostaglandin I2) is still the classical treatment of pulmonary hypertension. The pulmonary selectivity of these medications may be enhanced by inhalation. Bolus doses between 15 and 30 µg of inhaled prostacyclin have been administered in humans with potent effects on pulmonary pressure and oxygenation.<sup>89,90</sup> Inhaled NO and prostacyclin may have additive actions as both medications act through different vasodilatory pathways.<sup>91</sup>

Endothelin-1 is a potent vasoconstrictor released by endothelial cells in the pathophysiology of pulmonary hypertension. Bosenstan, a nonspecific endothelin-1 receptor antagonist, has been approved as a medication for primary pulmonary hypertension and will be available in the future.

#### **Conclusion**

Since the first use of iNO, its mechanisms of action have become clearer, and its indications have changed. Initially recognized as a pulmonary medication, data

indicate that the extra-pulmonary effects of iNO are significant and could be of clinical benefit. The mechanisms of iNO on pulmonary and extra-pulmonary inflammation are being studied and iNO could be an important adjuvant to modulate the inflammation that is induced by major surgery and trauma. The possible toxicity of iNO could be negated by medications such as antioxidants and reactive oxygen species scavengers.

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