DOI: 10.5114/kitp.2012.30848

Inhaled nitric oxide effects outside the lungs – proven and possible mechanisms

Tlenek azotu stosowany wziewnie – potwierdzone i prawdopodobne mechanizmy działania poza łożyskiem płucnym

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Kardiochirurgia i Torakochirurgia Polska 2012; 3: 352–356

Abstract

Inhaled nitric oxide has been shown to reduce pulmonary hypertension in several disease states. The vasodilatory effect of inhaled nitric oxide is limited almost exclusively to the pulmonary circulation, due to its rapid diffusion across the capillary membrane and its immediate deactivation by hemoglobin in the pulmonary vasculature lumen. However, many reports on the use of inhaled nitric oxide have revealed a broad spectrum of changes outside the lung. The remote effects are typically dose dependent and the mechanisms responsible for these effects are incompletely understood. New evidence indicates that nitric oxide inhalation leads to formation of new compounds which may be carried as thiol groups attached to protein in blood or act indirectly through nitrite metabolites. This review presents the mechanisms of inhaled nitric oxide conversion to active compounds/metabolites and discusses their actions beyond pulmonary circulation with special emphasis on the potential for systemic effects.

Key words: respiratory system agents, nitric oxide, nitrite, S-nitrosothiol, ischemia, reperfusion injury.

Streszczenie

Tlenek azotu stosowany wziewnie wykorzystywany jest w leczeniu różnych stanów chorobowych przebiegających z nadciśnieniem płucnym. Jego działania wazodylatacyjne ograniczone są wyłącznie do krążenia płucnego, ze względu na szybką dyfuzję przez błony kapilar płucnych i natychmiastową dezaktywację przez hemoglobinę. W ostatnim czasie jednak, pojawiło się wiele obserwacji wskazujących na szerokie spektrum działania wziewnego tlenku azotu również poza łożyskiem płucnym. Te odlegle systemowe efekty jego działania są zazwyczaj zależne od dawki, natomiast odpowiedzialne mechanizmy pozostają nie w pełni wyjaśnione. Wiele obserwacji wskazuje, że wdychanie tlenku azotu prowadzi do powstawania aktywnych S-nitrozotioli oraz nitrozylacji grup sulfhydrylowych różnych białek. Wszystko to w połączeniu z aktywnością produktów jego metabolizmu azotynów i azotanów, może stanowić potencjalnie bardziej stabilne źródło magazynowania tlenku azotu w ustroju. Prezentowana praca przedstawia mechanizmy konwersji tlenku azotu do aktywnych metabolitów i omawia ich działania poza łożyskiem płucnym, ze szczególnym uwzględnieniem potencjału działań ogólnoustrojowych w warunkach upośledzonej perfuzji narządowej.

Słowa kluczowe: tlenek azotu, uraz reperfuzyjny, azotyny, S-nitrozotiole.

Introduction

Inhaled nitric oxide (iNO) has been shown to reduce pulmonary hypertension associated with several disease states. It started to be applied in clinical work late in 1991, and became registered as a drug for use in persistent pulmonary hypertension of the newborn (PPHN) in the European Union in 2001. Inhaled nitric oxide therapy in neonates and children: reaching a European consensus in 2004 [1] and adults European Expert Recommendations in 2005 [2].

The therapeutic potential of inhaled NO as a selective pulmonary vasodilator was shown for the first time in a lamb model of pulmonary hypertension in 1991 [3]. The vasodilatory effect of inhaled nitric oxide is limited largely to the lungs, due to its rapid diffusion across the capillary membrane and its immediate deactivation by hemoglobin

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(Hb) in the pulmonary vasculature lumen. This is in contrast to intravenously infused vasodilators that can cause systemic vasodilation and severe systemic arterial hypotension. Inhaled NO therapy for selective pulmonary vasodilatation in adults and children has been recently reviewed by Bloch and Creagh-Brown [4, 5].

Many clinical and experimental reports on the therapeutic use of inhaled nitric oxide in various settings have revealed a broad spectrum of changes outside the lung [6]. The mechanisms responsible for these effects are incompletely understood [7]. The remote effects of iNO are typically dose-dependent and can take place in the absence of systemic hemodynamic changes. These effects go well beyond relaxation of vascular smooth muscle and include inhibition of leukocyte adhesion and migration and increases in renal glomerular filtration and natriuresis [8]. NO inhalation after various insults results in myocardial, central nervous system and liver function improvement. Additionally, multi-organ protection in sepsis was noted [9]. New evidence indicates that NO inhalation leads to formation of new compounds which may be carried as thiol groups attached to protein in blood [10]. Another possibility is that inhaled NO acts indirectly through nitrite and nitrate, metabolites which have been shown to elevate over time during exposure to inhaled NO [11]. A growing body of evidence indicates that iNO has no hemodynamic effects on normally perfused tissue, but increases blood flow selectively in ischemic tissue [12]. This review focuses on iNO effects outside the lungs, and discusses the possible mechanisms of action with particular attention to potential of the systemic effects of the gas in conditions of impaired organ perfusion.

Nitric oxide generation

Nitric oxide is produced endogenously in humans from the amino acid L-arginine by a family of enzymes known as nitric oxide synthase (NOS). The genes for the three different NOS isoforms – endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS) – are located on different chromosomes. eNOS was first discovered in the vascular endothelium and plays an important role in regulating vascular tone. nNOS was discovered in the brain and participates in central and peripheral neuronal physiology. iNOS was first identified in macrophages and plays an important role in infection. Transcription of iNOS is mainly driven by inflammatory agents such as cytokines and lipopolysaccharides. The different NOS may appear in almost any cell type. Binding of NO to the heme group of soluble guanylate cyclase (sGC) leads to increased conversion of GTP to cyclic guanosine monophosphate (cGMP), which in turn activates protein kinase G (PKG) [13].

Medical-grade NO gas is produced under carefully controlled conditions, diluted with pure nitrogen, and stored in the absence of oxygen. An iNO delivery system should allow for constant and accurate measurements of NO and nitrogen dioxide (NO_2). The measurement of iNO and NO_2 concentrations can be undertaken using chemiluminescence or electrochemical devices [14].

The biological action of inhaled nitric oxide

After inhalation, NO diffuses rapidly across the alveolar-capillary membrane into the subjacent smooth muscle of pulmonary vessels to activate sGC. The effects of cGMP are mediated through activation of its effector proteins – cGMP-dependent protein kinase PKG, cGMP-gated ion channels, and cGMP-regulated phosphodiesterases (PDE) [15]. The physiological action of cGMP is limited to its area of synthesis by its hydrolysis to GMP by cyclic nucleotide PDE or by its export from the cell. PDE5 is considered to be the most active cGMP-hydrolyzing PDE in smooth muscle. PDE5 has a high affinity for cGMP and is selectively inhibited by compounds such as zaprinast, sildenafil, and vardenafil [16].

Inhaled nitric oxide dilates pulmonary resistance vessels to improve ventilation–perfusion matching; iNO is therefore a selective pulmonary vasodilator. In the normal lung, a low oxygen tension constricts the vascular bed in hypoxic regions and redistributes blood flow toward lung regions with better ventilation and a higher intra-alveolar partial pressure of oxygen. Inhaled NO enhances this mechanism by increasing blood flow to well-ventilated lung areas that, in some diseases, have an elevated vasomotor tone. This vasodilatory effect of inhaled NO is in marked contrast to intravenously administered vasodilators. Such intravenous agents produce diffuse dilation of the pulmonary vasculature, including areas of non-ventilated lung, thereby increasing intrapulmonary shunting and reducing the PaO_2 [16]. Nitric oxide after inhalation diffuses into the bloodstream and is expected to react at a nearly diffusion-limited rate (1 s) with both oxy- and deoxyhemoglobin to form methemoglobin/nitrate and iron-nitrosyl-hemoglobin (HbFeIINO). The metabolic fate of iNO is similar to endogenous NO with the formation of nitrites and nitrates eliminated in the urine. Almost 70% of the inhaled gas will appear in the urine as nitrates within 48 hours of inhalation. Blood levels of nitrate have been reported to increase 4-fold during breathing of 80 parts/million (ppm) NO [17]. In addition to its pulmonary vasodilating effects, iNO has several other effects in the lung, including bronchodilatation, anti-inflammatory properties and anti-proliferative effects.

Observations indicating the systemic action of inhaled nitric oxide

Inhaled NO is a selective pulmonary vasodilator, but pioneering research already pointed to the possibility of discrete systemic effects of gas inhalation, particularly in terms of its higher concentrations of about 80 ppm [3]. In subsequent years there have been many observations supporting possibilities of multidirectional remote systemic iNO effects far beyond the pulmonary circulation. Studies in human volunteers showed that inhaled NO 80 ppm during blockade of regional NO synthesis can supply intravascular NO to maintain normal forearm blood flow and vascular function [17]. Inhalation of NO gas was shown to decrease systemic vascular resistance, decrease thrombosis after thrombolysis, decrease neointima forma-

tion after carotid artery injury, and cause bleeding time prolongation [18].

Inhaled nitric oxide adducts and metabolites in systemic circulation

Part of the intravascular NO which diffuses into vascular smooth muscle and activates sGC can escape hemoglobin scavenging and reacts with oxygen in plasma to form more stable NO adducts. Several NO-modified compounds have been proposed as species that function to preserve iNO bioactivity in blood. All these modified proteins can retain the biological properties of NO and are more stable than NO itself, thereby preserving and increasing the persistence of NO. Nitric oxide can form adducts with molecules containing sulfhydryl functional groups to yield S-nitrosothiols (SNO) such as S-nitrosocysteine (CysNO) and S-nitrosoglutathione (GSNO), which have been reported to possess NO-transporting activity [19]. SNO formation and stabilization occurs by endogenous NO-mediated nitrosylating agents such as dinitrogen trioxide (N_2O_3), or by transnitrosylation from low-molecular-weight SNO, such as GSNO or CysNO [20]. The S-nitrosylation of cysteine residue on proteins depends on the precise conditions of NO, O_2, hydrophobicity, nucleophilicity, and redox surrounding the targeted thiols and ultrastructural accessibility of cysteine residues under low-oxygen tension, such as hypoxia and ischemia, might determine whether a particular thiol in a given protein is subjected to S-nitrosylation [21].

NO and S-nitrosothiols in plasma can react with protein sulfhydryl, forming S-nitrosoproteins [22]. S-nitrosoalbumin (SNO-Alb) has been implicated in protection by iNO against reperfusion injury in systemic vessels, and possesses NO-like properties, including vasodilatation and inhibition of platelet aggregation [23]. The half-lives of SNO-Alb and GSNO have been reported to be 15-40 min and 8 min, respectively [24]. Additionally to SNO, a number of intravascular species capable of causing vasodilation have been found: nitrite N-nitrosamines, iron-nitrosyls and nitrated lipids [25]. In subsequent studies, S-nitrosylated proteins were expanded to include S-nitrosohemoglobin (SNO-Hb), which exhibits the ability to mediate selective vasodilation in proportion to the degree of hypoxemia. [10]. Inhaled NO can generate SNO-Hb through multiple reactions. Reactions with hemes of Hb to the adjacent Cysβ93 residue are relatively inefficient because of relatively low levels [10]. Much more effective is SNO-Hb generation with the involvement of GSNO produced in airways from N_2O_3, through a simple transnitrosylative transfer of the NO group. This pathway was suggested by Terpolilli and coworkers in their recently published experimental stroke model study. They have demonstrated neuroprotection by increasing circulating levels of SNO-Hb and the SNO-Hb–generating nitrite after NO inhalation [26].

Hemoglobin role in nitric oxide transport

A subject of very great interest is currently the dual role of red blood cells (RBCs) and hemoglobin, not only as carriers of oxygen but also as direct effectors of local blood flow. There have been suggested three main mechanisms by which RBCs can regulate their own distribution in the microcirculation: deoxygenation-dependent release of ATP from RBCs, which stimulates production of NO and other vasodilators in the endothelium; release of vasoactive NO from SNO-Hb upon deoxygenation; and reduction of naturally occurring nitrite to vasoactive NO by deoxygenated Hb [27]. Hemoglobin can react with nitric oxide and related compounds depending on specific conditions. NO can be consumed, bound, or generated by four different reactions:

- oxyhemoglobin + nitric oxide → methemoglobin + nitrate (the classical pathway),
- deoxyhemoglobin + nitric oxide ↔ nitrosyl-hemoglobin,
- Hb (β93-cys) + nitric oxide ↔ S-nitrosohemoglobin,
- deoxyhemoglobin + nitrite → methemoglobin + nitric oxide [28].

The O_2 binding and delivery properties of RBCs are guided by the allosteric properties of the hemoglobin inside the cells. Hemoglobin is in equilibrium between two quaternary structures, the relaxed (R) structure with high O_2 affinity, characterizing oxygenated Hb, and the tense (T) structure with low O_2 affinity, characterizing deoxygenated Hb. At high PO_2 (oxygen partial pressure), Hb will assume the R structure. As the blood enters the microcirculation, the PO_2 decrease will promote O_2 off loading from hemoglobin and a shift to the T structure [29]. Hemoglobin in the presence of oxyhemoglobin in its R structure binds NO at highly conserved Cys-β93 residues, forming SNOHb. Under physiologic conditions, SNOHb is produced in NO-Hb interactions in quantities and on time scales that compete favorably with those of methemoglobin and heme-iron nitrosyl hemoglobin, which were classically viewed as the terminal and sole products of reactions between NO and Hb [30]. Binding of NO to the heme iron of Hb predominates in the deoxygenated state on its T structure. As such, circulating erythrocytes may effectively store and release NO peripherally in areas of low oxygen tension, augmenting microvascular blood flow and oxygen delivery via hypoxic vasodilation of systemic vascular beds [7].

All these findings resulted in a model of the respiratory cycle, first proposed by Stamler and coworkers, which is based on the coordinated transport of three gases, NO, O_2, and CO_2. In this cycle, the delivery of NO bioactivity conveyed through SNO is coupled to O_2 delivery and thus is regulated by tissue PO_2 [10]. Hypoxia, hypercarbia, and acidosis promote the deoxygenated conformation (T-structure) in Hb that coordinately liberates SNO and O_2, thereby matching blood flow with metabolic demand [31]. More recently, hypoxic vasodilation has been shown in the absence of red blood cells, suggesting that other, possibly integrated, overlapping or redundant pathways exist to ensure tissue perfusion. These may include waste products of metabolism such as adenosine, potassium, lactate and/or carbon dioxide among others. In this context, plasma nitrite may provide a bridge between red blood cell dependent and independent effects [32].

Nitrite as a mediator of inhaled nitric oxide effects outside the lungs

Nitrate and nitrite generation has been reported to develop after NO inhalation and treatment with different NO donors including nitroglycerine [35]. For many years both nitrate and nitrite were considered as inert byproducts of nitric oxide metabolism. These observations were supported by studies from Lauer *et al*. [36], demonstrating that nitrite had no vasodilator activity when infused at concentrations of 200 µM in the forearm of normal human volunteers.

New evidence suggests that nitrite represents a circulating storage pool of NO and may selectively donate nitric oxide to hypoxic vascular beds [28]. Nitrite can be a substrate for NOS-independent generation of NO in vivo, and reduction of nitrite possibly occurs systemically in blood and tissues. There are several routes by which nitrite can be bioactivated to nitric oxide and other nitrogen oxides. In contrast to NOS-dependent nitric oxide production, these pathways are greatly enhanced during hypoxia and low pH. They may be considered as a backup system to ensure bioactive nitric oxide under conditions where NOS enzymes may be dysfunctional [13, 35].

The vasoactive role of nitrite was first shown by Cannon, who reported artery-to-vein gradients in nitrite across the human forearm, with increased consumption of nitrite during exercise stress, suggesting that nitrite was metabolized across the peripheral circulation. Additionally, in humans breathing 80 ppm iNO, the observed increase in peripheral forearm blood flow was only associated with increases in plasma nitrite and there was no significant increase in plasma SNO-albumin or erythrocyte SNO-Hb [17]. Mechanisms proposed for the in vivo conversion of nitrite to NO include enzymatic reduction by xanthine oxidoreductases (XOR) [36] and non-enzymatic disproportionation/acidic reduction. However, vasodilatation mediated by near-physiological concentrations of nitrite under normal physiological conditions appears to be inconsistent with a mechanism of nitrite reduction by XOR or disproportionate, because both of these pathways require very low pH and nearly anoxia. The observation that nitrite infusions produce vasodilatation along the physiological oxygen gradient suggests an alternative mechanism of bioactivation [37]. In the absence of molecular oxygen during hypoxia, NOS cannot produce NO and deoxyhemoglobin catalyses NO release from nitrite, thus potentially also providing hypoxia-specific vasodilatory effects. [28]. Other studies have revealed that nitrite-related RBC-dependent vasodilatation is initiated at an oxygen tension around hemoglobin P50 (arterial PO_2 of 40 mmHg) and occurs as hemoglobin unloads oxygen to 50% saturation. This maximal reductase activity of hemoglobin is regulated and peaks around the P50 level, because of two opposing chemical factors related to R or T hemoglobin conformation. R-state Hb exhibits a decreased redox potential of the hemes [38]. The maximal reductase activity around the P50 level involves the role of the T state or deoxygenated conformation of hemoglobin that has most hemes available for binding and reaction with nitrite. Such a maximal nitrite reductase activity at Hb P50 appears ideal for oxygen sensing and hypoxic vasodilation because this point is thermally, chemically, and electronically responsive to tissue metabolism. Additionally, a maximal reductase activity at P50 is biochemically consistent with a role in hypoxic vasodilation because physiological studies demonstrate an onset of hypoxic vasodilation at 40–60% hemoglobin oxygen saturation [39].

Hemoglobin, myoglobin, neuroglobin, XOR, aldehyde oxidase, carbonic anhydrase, eNOS, and mitochondrial enzymes have all been identified in nitrite bioactivation [25, 40]. Contribution from these factors varies between tissues and is dependent on local pH, oxygen tension, and redox status [13].

Conclusions

Several experimental and clinical studies have indicated that inhaled nitric oxide has no hemodynamic effects on normally perfused tissue, but increases blood flow selectively in ischemic tissue. Because of these properties iNO may be easy implemented as a rescue therapy for ischemic conditions in which collateral blood flow is important or until interventional or spontaneous reperfusion occurs. Numerous examples of iNO applications in various conditions of systemic organ dysfunction will be presented in the second part of this review.

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