Nitrite Signaling in Pulmonary Hypertension:Mechanisms of Bioactivation, Signaling, and Therapeutics

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Abstract

Significance: Pulmonary arterial hypertension (PAH) is a disorder characterized by increased pulmonary vascular resistance and mean pulmonary artery pressure leading to impaired function of the right ventricle, reduced cardiac output, and death. An imbalance between vasoconstrictors and vasodilators plays an important role in the pathobiology of PAH. Recent Advances: Nitric oxide (NO) is a potent vasodilator in the lung, whose bioavailability and signaling pathway are impaired in PAH. It is now appreciated that the oxidative product of NO metabolism, the inorganic anion nitrite (NO$_2^-$), functions as an intravascular endocrine reservoir of NO bioactivity that can be reduced back to NO under physiological and pathological hypoxia. Critical Issues: The conversion of nitrite to NO is controlled by coupled electron and proton transfer reactions between heme- and molybdenum-containing proteins, such as hemoglobin and xanthine oxidase, and by simple protonation and disproportionation, and possibly by catalyzed disproportionation. The two major sources of nitrite (and nitrate) are the endogenous l-arginine–NO pathway, by oxidation of NO, and the diet, with conversion of nitrate from diet into nitrite by oral commensal bacteria. In the current article, we review the enzymatic formation of nitrite and the available data regarding its use as a therapy for PAH and other cardiovascular diseases. Future Directions: The successful efficacy demonstrated in several animal models and safety in early clinical trials suggest that nitrite may represent a promising new therapy for PAH. Antioxid. Redox Signal. 18, 1797–1809.

Introduction

PULMONARY HYPERTENSION (PH) is a life-threatening progressive disorder characterized by persistent elevation in pulmonary artery pressure (>25 mmHg at rest), and increased pulmonary vascular resistance. With increasing pulmonary vascular resistance, there is a progressive increase in afterload on the right ventricle, leading to concentric hypertrophy, dilatation, and decreased function (Fig. 1). This exerts significant stress on the right heart that will eventually fail if left untreated, leading to a drop in cardiac output. Right heart failure and a low cardiac output lead to the major symptoms of pulmonary arterial hypertension (PAH), such as dyspnea on exertion and syncope, and increasing risk of death.

PAH is a multifactorial process, but early disease is related to a dysregulation of critical vasodilator pathways (down-regulation of nitric oxide [NO] and prostaglandin signaling) and vasoconstrictor pathways (upregulation of endothelin-1 and reactive oxygen species [ROS] signaling) (75). PAH is associated with decreased bioavailability and responsiveness of NO (14, 32). NO is produced in mammalian cells primarily in the metabolism of l-arginine. NO synthase (NOS) catalyzes the oxidation of l-arginine to produce l-citrulline in the presence of oxygen and NADPH (Fig. 2). Although the expression levels of endothelial NOS (eNOS) in patients with PAH can vary from high to normal, the formation of NO and eNOS activity have been found reduced in patients with idiopathic PAH (75). Recent studies suggest that eNOS uncoupling may relate to impaired NO production in PAH, a process by which the enzyme transfers electrons from the NOS reductase domain to the oxygenase domain and diverted to molecular oxygen forming superoxide rather than NO. This dysfunctional state of the eNOS enzyme in the presence of high levels of superoxide generation may increase the formation of peroxynitrite formation and enhance the vascular disease during PH.

Inhaled NO (iNO) is considered as a potential therapy targeting the NO pathway (33, 39), and a potent and selective pulmonary vasodilator (65). iNO therapy has been proposed for treatment of PAH, but also for persistent PH of the newborn (15), and bronchopulmonary dysplasia in prematurely born infants (21) and post-cardiac surgery (3). Other possible applications of iNO therapy include the treatment of pulmonary ischemia-reperfusion injury (9), the acute respiratory distress syndrome (18), and hypoxemia in the setting of severe
chronic obstructive pulmonary disease (26). However, iNO gas has its limitations regarding dose and duration of the exposure, cumbersome and expensive delivery systems, off-target reactions of NO with oxygen to form reactive nitrogen species, and possible rebound PH when the intervention is interrupted (38). These limitations open the door to other molecules that can be a source of NO and nitrosative signaling, including the anion nitrite (NO$_2^-$) (55). Nitrite is a unique reactive nitrogen species, as it is relatively stable compared to NO [51.4 min (67) vs. 0.05–1.8 ms (68) half-life in whole blood] and can readily be reduced to NO, oxidized to nitrogen dioxide, and protonated to form S-nitrosothiol, providing NO-dependent and NO-independent signaling effects that have been reported to modulate vascular remodeling (2, 86).

Rediscovering Nitrite in Biology

Although in the ancient Chinese medicine, the sodium salts of nitrate and nitrite were used as a remedy for heart pains and ischemia (circa 800 AD) (54), only until recently, nitrate (NO$_3^-$) and nitrite (NO$_2^-$) were mostly considered undesired residues in the food chain with potential carcinogenic activity. Nitrate salts have also been used by early civilizations to cure meats, which not only improve their storage time and produce their reddish color but also kill pathogenic bacteria (69). The mechanism of the meat preservation by nitrate was characterized in the 19th century, when it was discovered to be converted to NO (bound to myoglobin). During the 1970s, public concern over toxicity increased, when nitrate and nitrite were associated to the endogenous formation of N-nitrosamines, which are carcinogenic. While remaining extremely controversial, the epidemiological links between nitrate/nitrite exposure, for example, in high-nitrate foods such as leafy green vegetables, remain unclear (25, 79).

The first relationship between nitrite formation and NO production was found by studying immune responses in activated macrophages. Analyses of bacterial-infected (74) or tumor-bearing (36) mice showed that macrophages use L-arginine to produce nitrite and L-citrulline. This was the first demonstration of the oxidation of a terminal nitrogen atom of L-arginine to an inorganic nitrogen oxide, an enzymatic activity later shown to be mediated by inducible NOS (iNOS). Marletta and colleagues define that a terminal guanidino nitrogen atom was the precursor of nitrite and nitrate synthesized by activated macrophages (41), and Tannenbaum et al. (76) confirmed that nitrate and nitrite are formed de novo in the intestine of the human body, reflecting in vivo NO formation and oxidation. These observations were followed by the demonstration of NO synthesis by mammalian endothelial cells. Moving forward another decade, NO was identified as a critical regulator of vascular homeostasis, neurotransmission,

FIG. 1. Radiological imaging in PH. (A) Contrast enhanced-CT image showing enlarged pulmonary artery in PAH patient. (B) CT image obtained from patient with severe PH. The right-sided chambers are dilated, the right ventricle hypertrophied. (C) Apical four-chamber two-dimensional echocardiography image showing enlarged right-sided chambers and small left ventricle. CT, computed tomography; LA, left atrium; LV, left ventricle; PA, pulmonary artery; PAH, pulmonary arterial hypertension; PH, pulmonary hypertension; RA: right atrium; RV, right ventricle.
and host defense; and nitrite was considered a biomarker of NO levels or NO formation in vivo (40, 62).

More recently, increasing evidence suggests that nitrite not only is a biomarker of NO formation from NO synthesis but also represents a storage reservoir for NO that can be converted back to NO during physiological and pathological hypoxia (8, 16, 56). It is now known that nitrate and nitrite can be recycled to NO (or other bioactive forms of nitrogen) in blood and peripheral tissue (27, 55), representing an alternative to the classical L-arginine-NOS-NO signaling. In situations of hypoxia, when the oxygen-dependent function of the NOS may become compromised, nitrite reduction is enhanced. Chemically, nitrite shows a unique redox position between oxidative (NO2 radical) and reductive (NO radical) signaling and has a relatively long half-life in blood and tissue (67), representing a storage pool supporting NO signaling during hypoxic or metabolic stress.

The Nitrate-Nitrite-NO Signaling Pathway

The reductive mammalian nitrate-nitrite-NO pathway mediates important signaling events and complements the traditional oxidative L-arginine-NOS-NO pathway. The two major sources of nitrite (and nitrate) are the endogenous L-arginine-NO pathway and the diet. Our daily intake of exogenous nitrite mainly comes from food additives, preservatives, and drinking water, and nitrate from leafy greens and root vegetables. Upon intake of nitrate (Fig. 3), symbiotic bacteria in the oral cavity can reduce nitrate to nitrite producing a high concentration of nitrite (up to low millimolar) in saliva (8, 56). Salivary glands are able to concentrate and secrete nitrate (from plasma or diet), increasing the amount of nitrate available to the oral microbiome to be reduced to nitrite. When saliva is then swallowed and reaches the low pH milieu of the stomach, nitrite is converted nonenzymatically to NO via protonation and reduction (8) (Table 1). Nitrate and nitrite can be absorbed in the gastrointestinal tract into the circulation. Excess of nitrate will be excreted, and nitrite can be further reduced to NO by different enzymes through our body (55). NO can be oxidized to nitrate by oxyhemoglobin (29) or to nitrite by reaction with oxygen [hydrophobic NO autoxidation (53)] and by reaction with the copper-containing ceruloplasmin (73). This new pool of nitrite and nitrate is available again for uptake by the salivary glands, closing what is known as the enterosalivary circulation of nitrate (55).

Mechanisms of Nitrite Bioactivation

Nitrite can be reduced to NO in vivo by both enzymatic and nonenzymatic processes (Fig. 4). Biological non-enzymatic NO formation was first reported by Zweier et al. (87) in an ischemic rat tissue where, at low pH, there was NOS-independent NO production. Benjamin et al. (8) showed that in the highly acidic environment (pH 3) of the stomach, nitrite is converted to NO gas. Nitrite production of NO at acidic pH is also enhanced by the presence of reducing compounds such as copper, ascorbate, and polyphenols (82) [Table 1, Eqs. (1)–(3)].

Enzymatic NO formation from nitrite, via facilitated proton and electron transfer reactions, has been reported for a wide variety of metal-containing proteins, including hemoglobin (22), myoglobin (70), neuroglobin (77), cytochrome c (4), cytochrome c oxidase (12), eNOS (78), xanthine oxidoreductase (XOR) (84), aldehyde oxidase (AO) (50), and carbonic anhydrase (1).

Heme-containing proteins

Deoxyhemoglobin. Deoxyhemoglobin (deoxy-Hb) can catalyze nitrite reduction accompanied with NO generation and
production of ferric heme (Fe$^{3+}$). The formed NO can then bind to another deoxyheme to form iron-nitrosyl (Fe$^{2-}$NO) (Table 1) (22). As for other heme proteins, the reaction is faster at low pH, indicating the involvement of the protonated nitrite (nitrous acid—HNO$_2$) [Table 1, Eq. (6)]. The reaction is allosterically regulated, being slow in the T-state, but much faster for the R-state deoxy-Hb (37). There is a balance between the faster reaction of nitrite with the R-state oxyhemoglobin and the availability of deoxyhemes to bind to nitrite, leading to a maximal rate of nitrite reduction at about 50% hemoglobin–oxygen saturation. These factors have suggested that the reaction can respond to change in pH and oxygen tension in blood and tissues, and potentially mediate hypoxic vasodilation (28, 29, 37).

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**Cytochrome c oxidase (complex IV).** Nitrite can react with cytochrome c oxidase to form NO (12). This reductive reaction occurs between ferrous cytochrome c oxidase (Fe$_{2+}$) and nitrite and represents a typical electron–proton transfer reaction [Table 1, Eq. (6)]. Note that nitrite can also be reduced by other proteins, such as myoglobin, to form NO, which in turn can bind to ferrous cytochrome c oxidase and inhibit respiration. The formation of NO and inhibition of cytochrome c oxidase have been proposed as a pathway to reduce oxygen consumption at low oxygen levels, thus promoting oxygen diffusion deeper into ischemic tissues (35, 70, 71). In summary, cytochrome c oxidase has been proposed to function as both a nitrite reductase that generates NO, as well as a target for nitrite-NO-dependent regulation of respiration and oxygen consumption.

**Neuroglobin.** Evolved from a common ancestor shared by hemoglobin and myoglobin, human neuroglobin is found in brain neurons and also possess nitrite reductase activity. Neuroglobin, like cytochrome c, is a six-coordinate heme protein, with two histidines bound to the heme iron. The binding and reduction of nitrite to NO are favored when the heme is five coordinated. Tiso et al. (77) reported that deoxyneuroglobins where the sixth ligand (Histidine 64) is mutated to a nonheme-binding side chain (H64L and H64Q) can reduce nitrite at a very high rate. The wild-type neuroglobin six-to-five coordination is also regulated in a redox-dependent fashion, with oxidation of two surface redox-sensitive thiols to disulfides increasing the open probability of the heme and increasing the rate of nitrite binding and reduction to NO. Tiso et al. showed in these studies that the nitrite reductase reaction of neuroglobin could modulate mitochondrial respiration, similar to that shown for myoglobin (Table 2) (77). This pathway suggests a possible redox sensor mechanism for neuroglobin-mediated nitrite reduction. (Fig. 6)

**Nitric oxide synthase.** While though NOS is responsible for normoxic NO generation from arginine oxidation, it has been recently reported that NOS also catalyzes nitrite reduction to NO under anoxic conditions. Vanin et al. reported that eNOS catalyzes anoxic NO formation in murine microvascular brain endothelial cells, which is abolished by the eNOS

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**Table 2. Summary of Bimolecular Reaction Rates of Heme-Containing Proteins with Nitrite**

<table>
<thead>
<tr>
<th>Protein</th>
<th>$k$ (M$^{-1}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (T-state)$^a$</td>
<td>0.12</td>
</tr>
<tr>
<td>Hemoglobin (R-state)$^a$</td>
<td>6</td>
</tr>
<tr>
<td>Myoglobin$^b$</td>
<td>5.6</td>
</tr>
<tr>
<td>Neuroglobin ($\sim$SS)$^c$</td>
<td>0.12</td>
</tr>
<tr>
<td>Neuroglobin ($\sim$SH)$^d$</td>
<td>0.062</td>
</tr>
</tbody>
</table>

$^a$Human, at 37°C, pH 7.4 (37).

$^b$Sperm whale, at 25°C, pH 7.4 (77).

$^c$Human, at 25°C, pH 7.4 (77).
FIG. 6. Neuroglobin acts as a nitrite reductase under oxidative stress conditions. The thiol state of the glutathione is a good indicator of the oxidative stress in vivo conditions and can be modulated in vitro. In normal conditions, cells keep a high concentration of reduced glutathione (GSH) and low oxidized glutathione (GSGG). In these circumstances, the disulfide bond of Ngb is not formed, and the protein has a low nitrite reductase activity (left). As oxidative stress conditions develop (right), reduced glutathione is consumed, and the number of neuroglobin molecules with formed disulfide bonds increases. This leads to increased production of NO from nitrite, causing the inhibition of respiratory enzymes and limiting oxygen consumption and reactive oxygen species-producing reactions [reproduced with permission from (77)]. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)

inhibitors NO-nitro-L-arginine or NO-nitroarginine methyl ester (L-NAME) (78). This pathway may represent an alternative NO source during severe hypoxia in tissues. Webb et al. have shown that eNOS exists on whole red blood cells (81). When nitrite reacted with red blood cells in the presence of L-NAME or L-arginine at low oxygen tensions, NO production was inhibited by about 60%, while d-arginine, the inactive isomer, showed no significant effects, leading them to propose that this pathway may have some relevance to vasodilation. Nevertheless, further studies are needed to reconcile how this pathway may function in vivo, because it requires extremely low oxygen tensions.

Molybdopterin-containing proteins

XOR and AO are two dimeric molybdopterin enzymes ubiquitously expressed in human tissues, containing one molybdenum (Mo) center, two nonidentical Fe2S2 clusters, and a flavin adenine dinucleotide cofactor. XOR catalyzes the terminal two steps in purine degradation and exists in cells primarily as a dehydrogenase (xanthine dehydrogenase [XDH]) where substrate-derived electrons reduce NAD+ to NADH. During inflammatory conditions, oxidation of critical cysteine residues or limited proteolysis converts XDH to xanthine oxidase (XO) (80). XO transfers substrate-derived electrons to O2, generating O2·− and H2O2. However, conversion to XO is not a requisite for ROS production, as XDH displays partial oxidase activity under conditions in which the NADH/NAD+ ratio is increased such as the hypoxemia (34). It is also under hypoxic (0–1% O2) conditions that both XO- and XDH-mediated nitrite reductase activities have been reported (47, 51). This is evidenced by inhibition of XO- and XDH-mediated reduction of nitrite to NO by the XOR-specific inhibitor, oxypurinol, and/or high concentrations of xanthine. This oxypurinol- and xanthine-induced inhibition of NO generation rates results from their binding to the Mo site, which is also the catalytic center for nitrite binding and reduction. A similar effect on nitrite reduction was observed for AO, which is inhibited by raloxifene. As the inhibitor for the flavin site, diphenylene iodonium chloride does not affect nitrite reduction when xanthine or aldehyde served as the electron donor, but considerably reduces rates of NO formation rates when NADH is the reducing substrate. These experiments indicate that nitrite is reduced at the Mo center of XO or AO, but electrons can be provided either at the Mo or the flavin site by various substrates (Fig. 7). It also suggests that electron withdrawal from XOR or AO by nitrite may serve not only to produce NO but also to reduce ROS formation and as such elicit salutary actions regarding NO derived from alternative sources.

Nitrite disproportionation

Another mechanism for nitrite bioactivation is disproportionation, with nitrous acid or NO− reacting with nitrite to form N2O3 [Table 1, Eqs. (2) and (3)]. This reaction has been proposed to be catalyzed by ferric hemoglobin (5) as well as carbonic anhydrase. Aamand et al. (1) recently demonstrated

FIG. 7. The nitrite reductase activity of XOR and AO can be inhibited at either molybdenum or flavin site. Oxypurinol or high concentrations of xanthine inhibit nitrite reduction by binding to the molybdenum center of XOR. AO can be inhibited specifically by raloxifene, which binds to the molybdenum site. Electron can be provided at the flavin site, which is transferred via Fe2S2 clusters to the molybdenum site. DPI blocks the electron transfer at the flavin site, thus inhibiting the nitrite reductase activity of XOR or AO. The relative distances of the four redox-active centers were taken from the crystal structure of the bovine XOR (PDB code: 1fo4). AO, aldehyde oxidase; DPI, diphenylene iodonium chloride; XOR, xanthine oxidoreductase. (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)
that NO was generated by nitrite reactions with carbonic anhydrase under both normoxia and hypoxia, which was stimulated by dorzolamide and acetazolamide, two specific inhibitors of carbonic anhydrase (Table 3). They propose that nitrite is structurally similar to bicarbonate and may react with histidines in the enzyme catalytic site to disproportionate to NO. However, more work is needed to fully explore this newly described pathway.

**Therapeutic Application of Nitrite for PH**

Recent studies have investigated the therapeutic effects of nitrite in PH models (85). Hunter et al. (38) first reported the effects of nebulized nitrite as a selective pulmonary vasodilator in an ovine hypoxia model. In this study, vasoconstriction induced by hypoxia was reduced by inhalation of NO gas (20 ppm) or nebulized nitrite (300 mg in 5 ml of phosphate-buffered saline for 20 min). Nebulized nitrite significantly reduced the pulmonary arterial pressures with lesser effects on systemic mean arterial blood pressure. The kinetic therapeutic responses to nitrite and NO were very different: The nitrite response was slower and lasted longer (over 60 min after inhalation), whereas the effect of NO response was faster, but returns rapidly to the hypoxic baseline when the treatment was discontinued (Fig. 8). The nitrite effect appears to be mediated by its slow conversion to NO, since measurements of exhaled NO gas increased, with values above baseline maintained after the end of the treatment. In addition, consistent with a possible role of deoxymyoglobin as the nitrite reductase protein, formation of iron-nitrosyl-hemoglobin was also reported (38). More recently, using an ovine model of hemolysis-induced PH, halted its progression and reversed high right ventricular pressures. In the monocrotaline-induced PAH rat model, nebulized nitrite was able to diminish monocrotaline-induced muscularization and hyperplasia of the small pulmonary arteries. Nitrite effects in both models were blocked by the XO inhibitor allopurinol (in vitro) and by tungsten-enriched diet (in vivo), which replaces Mo in the active sites of enzymes such as XO and AO, inhibiting their activity. These data suggest that the protective effect of nitrite against the development and progression of hypoxia-induced PAH is mediated by XOR (86). Additional work suggested that the nitrite effect was mediated by NO-cyclic guanosine monophosphate signaling and downstream induction of the cell cycle checkpoint inhibitor p21, which inhibits smooth muscle cell proliferation (Fig. 9).

Intravenous administration of sodium nitrite has been also explored in the context of PH. Casey et al. (11) investigated the effect of intravenous sodium nitrite (10–100 μmol/kg) as a pulmonary vasodilator in rats under hypoxic conditions. A vasodilatory effect in both pulmonary and systemic arterial pressure was observed, and these effects were inhibited by allopurinol, consistent with other rodent PAH models (86). While these studies suggest a role for XO as a nitrite reductase in rodent models, human studies have been performed testing a role for NO in nitrite-dependent vasodilation. Infusions of oxypurinol for 30 min, followed by coinjections of nitrite, did not block nitrite-dependent vasodilation in humans (17). Paradoxically, oxypurinol increased nitrite-dependent vasodilation by about 10%. Future studies are required to reconcile findings in rodent disease models compared with human physiological studies.

**Other Therapeutic Opportunities for Nitrite in Vascular Disease**

In any pathological condition where NO bioavailability is compromised, nitrite administration may provide a therapeutic approach to restore NO levels through the nitrate-nitrite-NO signaling pathway. This approach has a direct relevance for cardiovascular disease, given the well-known vasodilatory effect of NO, and now appreciated vasodilatory effects of nitrite. We will summarize here some of the current evaluations of nitrite in vascular disease.

**High blood pressure**

As discussed earlier, plasma nitrite levels can be modified by dietary nitrate intake. Larsen et al. (49) studied the effect of dietary nitrate supplementation on healthy volunteers and found a significant reduction in blood pressure and increases in plasma nitrate and nitrite. Other studies have confirmed that
dietary nitrate can decrease arterial blood pressure and enhance other parameters linked to NO metabolism, such as platelet aggregation and endothelial function during ischemia. Interestingly, these effects may be greater in men than that in women (46). In general, the dietary effects of nitrate are comparable to effects of vegetable-rich diet, suggesting that the high nitrate content of vegetables can be a part of their observed effects on blood pressure (59). Low doses of intraperitoneal, inhaled, or oral sodium nitrite have been shown to generate NO in blood vessels and to inhibit proliferative responses of smooth muscle cells in murine models of carotid injury.

**Angiogenesis**

Vascular endothelial growth factor-induced proliferation and organization of human endothelial cells have been shown...
to be dependent on eNOS and NO production (63), suggesting that nitrite-based therapies could be used to promote angiogenesis. Consistently, chronic intravenous administration of sodium nitrite has been shown to induce angiogenesis in a mouse model of hindlimb ischemia (48), and sodium nitrate therapy stimulated ischemic vasodilation and angiogenic activity after permanent femoral artery ligation (64).

**Sickle cell disease**

The pathology of sickle cell disease relates to vaso-occlusion by sickled erythrocytes and vasoconstriction due to increase NO scavenging associated with hemolytic anemia (83). Patients with sickle cell disease develop PH as they age, suggesting a potential role for nitrite therapy. A phase Ib clinical study by Mack et al. has shown that infused sodium nitrite was safe and increased forearm blood flow in patients with sickle cell disease (57), although sickle cell patients show a somewhat reduced response to nitrite as compared to healthy volunteers (16, 58).

**Myocardial ischemia**

Nitrite has protective effects in different ischemia reperfusion injury models (19). The effects are at least partly due to the ability of nitrite to reversibly inhibit mitochondrial metabolism, via S-nitrosation of complex I, and reduce reperfusion ROS formation (20, 60, 72). This reduction in reperfusion ROS is associated with a prevention of opening of the mitochondrial permeability transition pore and release of cytochrome c (72) (Fig. 10). Several studies have focused on the effects of nitrite treatment on myocardial ischemia. Duranski et al. showed a large effect in decrease of the infarct size when nitrite was applied during ischemia (23). The beneficial effect of nitrite in cardiac infarct has been found be independent of the time of administration during the ischemic phase and can be obtained by dietary intervention (31). The use of intravenous sodium nitrite to ameliorate the consequences of acute myocardial infarction is currently being evaluated in phase I–II clinical trials.

**Stroke**

Several studies have investigated the use of nitrite on stroke therapy in preclinical models. Early administration of nitrite during reperfusion shows favorable effects in rats (42), although responses depended on the nitrite dosage. High amounts of nitrite provided no protection, and low doses reduced the infarction size and enhanced local cerebral blood flow and functional recovery (44). Long-term, high-dose (100 μg/kg) nitrite treatment showed improvement in the recovery after stroke in a rat ischemia model (45). Some positive effects have been also observed in a rat intracranial hemorrhage model (43).

Nitrite therapy has also been applied in the treatment of delayed cerebral vasospasm after subarachnoid hemorrhage, a subtype of hemorrhagic stroke. This condition is characterized by decreased middle cerebral artery flow due to the narrowing of large-capacitance arteries and often develops days after subarachnoid hemorrhage. Several studies indicate that the vasospasm is related to decreased bioavailability of NO, at least in part due to inhibition of eNOS and NO scavenging by hemoglobin from the subarachnoid clot. Pluta et al. (66) studied the effect of nitrite in a primate model of subarachnoid hemorrhage, and showed that nitrite prevented the development of cerebral vasospasm.

**Therapeutic Developments**

Nitrite has been proposed and tested successfully in several animal models and patients, with a variety of delivery

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**FIG. 9.** Nitrite-induced SMC proliferation is dependent on XOR. Hypoxia-induced PAH is inhibited by NO generation from nitrite, which is catalyzed by XOR. NO production from nitrite increases expression of p21, which inhibits SMC proliferation. Allopurinol or tungsten diet treatment inhibits XOR activity (85, 86).

**FIG. 10.** Nitrite mediates cytoprotection in ischemia/reperfusion injury. Nitrite potently mediates cytoprotection after ischemia/reperfusion (I/R) through the transient inhibition of complex I (via S-nitrosation) and subsequent limitation of oxidative damage. Because oxidants have been shown to sensitize the permeability transition pore, cytoprotective effects of nitrite could in part be caused by nitrite-dependent protection against pore opening after I/R (72). (To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.)
systems and formulations. A mixture of sodium nitrite and acidifying agents such as ascorbic acid can rapidly release NO. This topical administration has been evaluated for its antimicrobial activity in various skin infections (61) and, with some modifications, in preventing catheter-associated urinary tract infections (10). It is also well established that nitrite can be given orally, but its bioavailability can be difficult to assess, because it is extremely dependent on the oral microbiome and its variable metabolism within the gastrointestinal tract. However, the use of organic allylic nitrocompounds as nitrite donors may overcome this issue (13). A third possibility is the much anticipated use of intravenous infusions of nitrite, but the dose and the duration of the treatment still need to be adjusted (67). One of the adverse effects of inorganic nitrite is the formation of methemoglobin. Nitrite can oxidize the iron at the heme group, modifying it from a ferrous (Fe²⁺) oxygen-binding form to a ferric (Fe³⁺) nonoxygen-binding state. If the increase of methemoglobin in circulating blood is higher than 5%, it can lead to cyanosis. However, studies with nitrite intravenous studies only report undetectable or modest increase (67).

As described above, inhaled sodium nitrite is a pulmonary vasodilator that can effectively prevent or reverse PAH in animal models. Studies suggest that it can be delivered safely, and it is ready for clinical translation for PAH patients. Animal toxicology studies with inhaled nitrite in rodents and dogs have been completed and phase Ia and Ib studies in normal volunteers completed. In a phase Ia study, inhaled nebulized sodium nitrite, at doses >17 mg, increases exhaled NO, whereas methemoglobin levels remained <3.5% in all subjects (Bradley et al., unpublished data). A proof-of-concept phase II trial of inhaled nitrite in patients with PAH is currently enrolling sites in the United States and Europe.

Summary and Conclusions

PAH is associated with decreased bioavailability and responsiveness of NO. Despite the potential therapeutic effect of iNO as a selective pulmonary vasodilator, its administration is inconvenient and difficult. The discovery that nitrite is a naturally occurring molecule in the body and may act endogenously as a reservoir of NO has suggested the idea that this anion may represent an alternative strategy for an effective NO-based therapy. NO can only be administered as a gas and cannot be mixed with oxygen, or it reacts to NO₂. Nitrite (as a soluble salt) can be effectively delivered as a nebulized liquid, dry powder, intravenous solution, or oral formulation. Also, when compared with INO, nitrite has a longer half-life. However, two caveats arise for either iNO or nitrite therapies: (i) both may have effects in conditions with soluble guanylate cyclase dysfunction (oxygenation or downregulation); and (ii) potential harmful side products may be formed by reaction with ROS (NO₂⁻, peroxynitrite, or methemoglobin formation). Nitrite has demonstrated efficacy in multiple animal models not only of cardiovascular disorders but also of inflammatory diseases and bacterial infections. Clinical trials are ongoing and more planned in the near future to demonstrate the therapeutic efficacy of nitrite in patients with PAH.

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Author Disclosure Statement

Dr. Gladwin is listed as a coinventor on an NIH government patent for the use of nitrate salts in cardiovascular diseases. Dr. Gladwin consults with Aires Pharmaceuticals on the development of a phase II proof-of-concept trial using inhaled nitrite for pulmonary arterial hypertension.

References

11. Casey DB, Badejo AM, Jr., Dhandilal JS, Murthy SN, Hyman AL, Nossaman BD, and Kadowitz PJ. Pulmonary vasodilator responses to sodium nitrite are mediated by an
The reference text in the image is about nitrite signaling in pulmonary hypertension. It discusses various studies on the role of nitrite in pulmonary function, including its effects on hypoxic conditions, mitochondrial cytochrome oxidase, and its potential as a therapeutic agent. The text cites numerous studies and authors, such as Egemnazarov B, Schermuly RT, Dahal BK, Elliott GT, Ho-glen NC, Surber MW, Weissmann N, Grimminger F, Seeger W, and Ghofrani HA. The document also mentions the importance of nitrite in the context of hypoxic signaling in eukaryotes and its implications for oxygen sensing.

Some key points from the reference text include:

- The role of nitrite in the regulation of mitochondrial function and nitric oxide production.
- The potential of nitrite as a therapeutic agent in pulmonary hypertension.
- The study of nitrite signaling in hypoxic conditions and its relationship to oxygen sensing.
- The importance of nitrite in the context of hypoxic signaling in eukaryotes.

The document is a valuable resource for researchers and clinicians interested in the mechanisms of pulmonary hypertension and the potential therapeutic applications of nitrite.

The reference text is a comprehensive review of the current understanding of nitrite signaling in pulmonary hypertension, highlighting the latest findings and future directions in this field.


72. Shiva S, Wang X, Ringwood LA, Xu X, Yuditskaya S, Annavajhala V, Miyajima H, Hogg N, Harris ZL, and Gladwin MT. Ceruloplasmin is a NO oxidase and nitrite synthase that...


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Abbreviations Used

AO = aldehyde oxidase
cGMP = cyclic guanosine monophosphate
CT = computed tomography
dehoxy-Hb = deoxyhemoglobin
DPI = diphenylene iodonium chloride
eNOS = endothelial NOS
GSNO = S-nitroso glutathione
iNO = inhaled NO
iNOS = inducible NOS
LA = left atrium
L-NAME = Nω-nitroarginine methyl ester
LV = left ventricle
Mo = molybdenum
NO = nitric oxide
NOS = nitric oxide synthase
PA = pulmonary artery
PAH = pulmonary arterial hypertension
PH = pulmonary hypertension
RA = right atrium
ROS = reactive oxygen species
RV = right ventricle
sGC = soluble guanylate cyclase
SMC = smooth muscle cell
XDH = xanthine dehydrogenase
XO = xanthine oxidase
XOR = xanthine oxidoreductase