

Nitric Oxide Therapeutics in Pulmonary Vascular Disease

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Clinical trials of inhaled nitrite to treat pulmonary arterial hypertension (PAH) are ongoing. The pathogenesis of PAH involves smooth muscle hypertrophy as a result of endothelial dysfunction and/or chronic vasoconstriction. Nitric oxide (NO) is a potent vasodilator and a primary therapeutic pathway for PAH. This review will address some of the emerging PAH treatment strategies utilizing the NO pathway.

The pathogenesis of pulmonary arterial hypertension (PAH) involves smooth muscle hypertrophy as a result of endothelial dysfunction and/or chronic vasoconstriction. Progressive vessel remodeling occurs with intimal and adventitial proliferation.¹ Nitric oxide (NO) is a potent vasodilator and a primary therapeutic pathway for PAH.² Flow through the vessel produces shear stress on the endothelium, activating nitric oxide synthase (NOS), which converts L-arginine to L-citrulline and NO. NO then diffuses into the vascular smooth muscle cells to activate soluble guanylyl cyclase, which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), mediating vasodilation via increased activity of calcium-sensitive K⁺ channels (BK_{Ca}).³ This inhibits voltage-gated, L-type Ca²⁺ channels, with subsequent decreased intracellular Ca²⁺, resulting in vasorelaxation (Figure 1).⁴

NO PATHWAY THERAPEUTIC TARGETS FOR PAH

There is therapeutic opportunity at each step along the NO pathway (Figure 2). Working through the pathway, L-arginine can be given to load the conversion to L-citrulline and NO, NO can be given directly or as nitrite, soluble guanylate cyclase can be directly stimulated by riociguat, phosphodiesterase type 5 can be inhibited by sildenafil or

tadalafil to increase cGMP, and calcium channels can be directly blocked by calcium channel inhibitors.

Administration of L-arginine has been shown in very small studies to reduce mean pulmonary artery pressure and pulmonary vascular resistance.^{5,6} This has been associated with increased levels of L-citrulline, indicating production of NO. However, there has not been enough evidence to include it in the most recent guidelines.^{7,8}

NO can be given directly via inhalation. It is a mainstay of the acute vasodilator challenge when invasively measuring hemodynamics. Because of its extremely short duration of action, NO must be given continuously for therapeutic benefit. This, as well as cost, has limited therapeutic administration of inhaled NO to short courses in the intensive-care setting, where acute reduction of pulmonary pressures may benefit pulmonary hypertension (PH), acute respiratory distress syndrome, pulmonary embolus, and perioperative right ventricular (RV) failure.⁹⁻¹² Unfortunately, inhaled NO has shown no improvement in mortality.^{13,14}

The soluble guanylate cyclase stimulator riociguat has recently shown benefit in both PAH and chronic thromboembolic PH (CTEPH).^{15,16} Significant improvements were seen in multiple endpoints including hemodynamics (pulmonary vascular resistance, mean

pulmonary arterial pressure, and cardiac output), functional class, quality of life, and clinical events (hospitalizations due to PH, start of new treatment for PH, decrease in 6-minute walk distance [6MWD] due to PH, worsening of functional class due to PH, death; seen only for PAH, not CTEPH, although there were few numbers of events).

The phosphodiesterase type 5 inhibitors, sildenafil and tadalafil, are mainstays of therapy for PAH, with improvements seen in hemodynamics (pulmonary vascular resistance, mean pulmonary arterial pressure, and cardiac output), functional class, 6MWD, and remarkable survival results.¹⁷⁻²⁰ Beyond the effects on the pulmonary circulation, sildenafil has been reported to improve RV function,²¹⁻²⁴ which is now felt to be an important goal of PAH treatment due to the implications of RV failure on morbidity and mortality.²⁵⁻²⁹

Calcium channel blockers were among the first medications used to treat PAH. Nifedipine, diltiazem, and amlodipine have been used. There are several limitations to calcium channel blockers. Positive clinical response to therapy requires high doses that are difficult to achieve, and lack of durable treatment response limits the therapeutic benefit of calcium channel blockers.^{30,31}

LIMITATIONS TO NOS-DEPENDENT NO SIGNALING

There are several limitations to NOS-dependent NO signaling. First, activity is limited by the short half-life of NO in blood (<2 milliseconds because NO is

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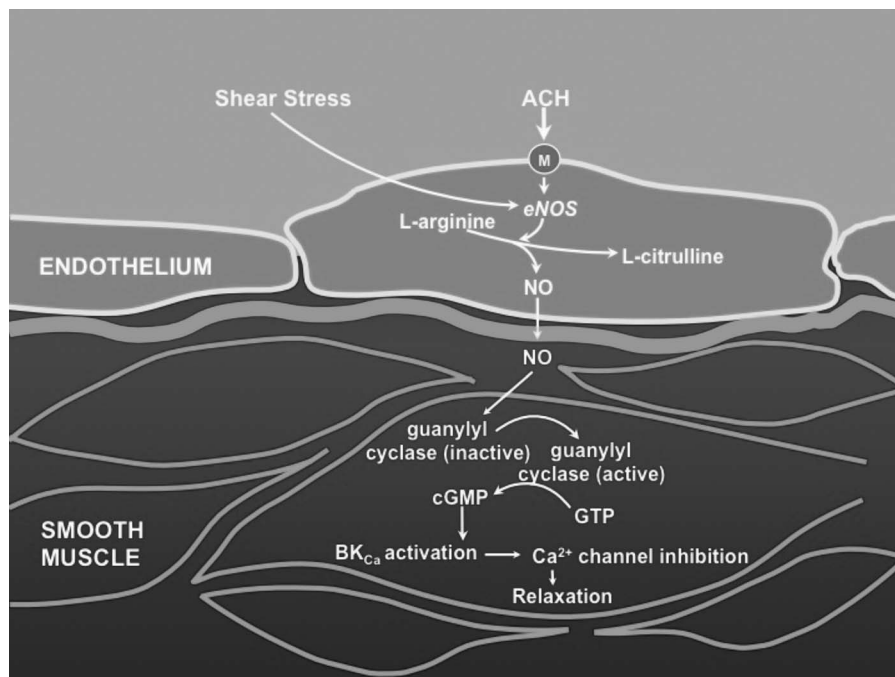


Figure 1: Nitric oxide pathway. NO, nitric oxide; ACH, acetylcholine; eNOS, endothelial nitric oxide synthase; cGMP, guanosine monophosphate; GTP, guanosine triphosphate; BK_{Ca}, calcium-sensitive K⁺ channels.

scavenged by hemoglobin). Next, there is a lack of an intrinsic mechanism for hypoxic response since endothelial NOS (eNOS) requires a molecule of oxygen to form NO. Finally, NOS is uncoupled and fails to generate NO with inflammatory and oxidant stress, as occurs in disease.

NITRITE AS A SOURCE OF NO TO TREAT PAH

There is another pathway to form NO. The NOS-independent pathway reduces nitrite (NO₂⁻) and nitrate (NO₃⁻) to NO. Once nitrite is formed, there are numerous pathways to reduce to NO, ranging from hemoglobin and myoglobin to xanthine oxidoreductase, ascorbate, polyphenols, and protons. Conversely, NO can be oxidized to nitrate and nitrite in blood and tissue, where it can be transported to be reduced back to NO as needed. Thus, nitrite and nitrate can be viewed as storage pools for NO activity, complementing the NOS-dependent pathway. Even more important in disease, these pathways are upregulated during hypoxia and acidosis, providing NO at times when NOS enzyme activity is impaired.³²

Nitrite has been shown to be an intrinsic vasodilator. In 10 healthy sub-

jects, infusion of sodium nitrite into the brachial artery increased blood flow.³³ This increase in blood flow was not blocked by simultaneous infusion of L-NMMA to inhibit NOS, indicating a

separate vasodilatory mechanism from NOS. This study also showed nitrite formation under hypoxic conditions, with an inverse relationship of iron-nitrosyl-hemoglobin formation to oxygen saturation. This occurs via a 2-step process in which nitrite is reduced by deoxyhemoglobin to NO and then NO combines with deoxyhemoglobin to form iron-nitrosyl-hemoglobin:

1. NO₂⁻ (nitrite) + HbFe^{II} (deoxyhemoglobin) + H⁺ → HbFe^{III} (methemoglobin) + NO + OH⁻
2. NO + HbFe^{II} (deoxyhemoglobin) → HbFe^{II}-NO (iron-nitrosyl-hemoglobin)

Thus, iron-nitrosyl-hemoglobin is indicative of NO formation from nitrite and occurs under hypoxic conditions.^{34,35}

While hemoglobin-related reduction of nitrite to NO appears to play a major role in vasodilation, there are multiple other pathways. Molybdopterin reductases (xanthine oxidase, aldehyde oxidase, sulfite oxidase, mitochondria amidoxamine reducing component) are in tissue such as myocardium as well as mito-

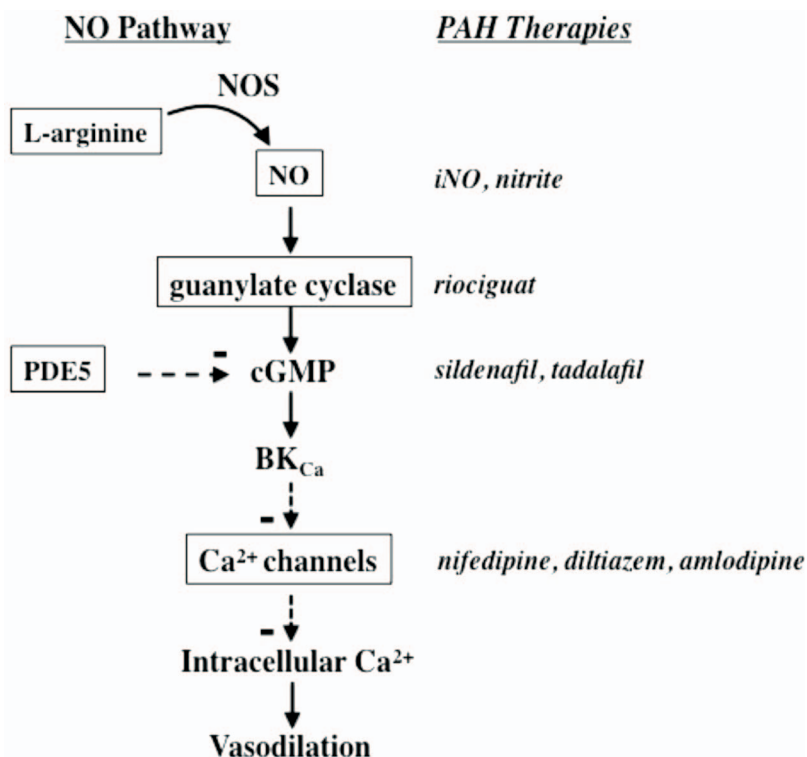


Figure 2: Nitric oxide pathway therapeutic targets. NO, nitric oxide; PAH, pulmonary arterial hypertension; iNO, inhaled nitric oxide; PDE5, phosphodiesterase type 5; cGMP, guanosine monophosphate.

chondria. Nitrite anhydrases (ferric hemoglobin, nitrophorin, carbonic anhydrase) can stabilize reactions effectively, allowing for NO transport.

In terms of treatment of PAH, nitrite has been shown in preclinical models to ameliorate hypoxia-induced pulmonary vasoconstriction and prevent RV hypertrophy.^{36,37} Inhaled nitrite is currently under development for the treatment of PAH, with data to date showing safety and initial efficacy trials underway. There has been no significant methemoglobinemia by 1- and 12-month rat and canine toxicology studies, a concern raised by the chemistry shown above, except at very high doses. A repeat-dose escalation Phase 1A study has been completed in normal volunteers showing safety up to 90 mg (dose placed into the medication chamber) on background sildenafil in normal volunteers and 11 PAH patients. A Phase 2 proof of concept international trial of chronic administration of inhaled nitrite 4 times per day was closed earlier this year for financial reasons, but prior to this, 29 PAH patients were treated for up to 354 days (information courtesy of Aires Pharmaceuticals). A Phase 2 study of the acute effects of inhaled nitrite on PH patients (both Group 1 and Group 3) is in progress at our institution in which there have been no adverse events, and preliminary results suggest significant reduction in pulmonary vascular resistance to a similar degree as inhaled NO, a modest but significant decrease in systemic vascular resistance, a significant decrease in right atrial pressure, and an increase in RV contractility index.

In summary, clinical trials of inhaled nitrite to treat PAH are ongoing, with exciting early results that, if confirmed in future larger trials, may broaden treatment strategies utilizing the NO pathway.

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