Pulmonary arterial hypertension (PAH) is a devastating and life-threatening clinical syndrome characterized by elevated pulmonary artery pressures leading to progressive symptoms, including shortness of breath, fatigue, and a decline in functional ability. The hemodynamic definition of PAH is a mean pulmonary artery pressure of >25 mm Hg at rest or >30 mm Hg during exercise, with a normal pulmonary arterial wedge pressure ≤15 mm Hg.1 Recent data from a French registry suggests that the prevalence of PAH is about 15 cases per 1 million,1 and the Registry to Evaluate Early and Long-Term Pulmonary Arterial Hypertension Disease Management (REVEAL) demonstrates that the mean age at diagnosis is 48 years and that mostly women (80%) are affected.2 The pathophysiology of PAH includes endothelial dysfunction, vascular remodeling with progressive obstruction and obliteration of pulmonary arteries, and ultimately, right atrial pressure, right ventricular hypertrophy, right ventricular failure, and death.1 Endothelial dysfunction is believed to be an early event in PAH, and is characterized by overproduction of vasoconstrictor/mitogenic compounds, such as endothelin and thromboxane A2, and by insufficient production of vasodilators, such as prostacyclin and nitric oxide (NO). These observations led to the development of 3 different classes of therapies that are currently in clinics, either alone or in combination: prostacyclin analogs, endothelin receptor antagonists, and phosphodiesterase (PDE) inhibitors. Although current therapies can improve symptoms and reduce severity of the hemodynamic disorders, gradual deterioration of pulmonary and cardiac functions often necessitates lung transplantation. The prognosis of PAH is reportedly poor, with ≈15% mortality within 1 year of modern therapy.1 Therefore, a renewed interest has been focused on the mechanisms of PAH pathogenesis to identify a novel therapeutic target.

It is now well established that disruption or dysfunction of the endothelium promotes vascular lesion formation. This is due in part to the loss of endothelial vasoprotective factors such as NO that promote endothelial survival and proliferation and inhibit platelet aggregation and vascular smooth muscle cell proliferation.3 In the vasculature, NO is derived from the oxidation of L-Arginine, catalyzed by the constitutively expressed enzyme, endothelial nitric oxide synthase (eNOS).4 This endothelial-derived NO diffuses from the vascular endothelium into the smooth muscle cell layer, where it activates soluble guanylate cyclase leading to smooth muscle relaxation.4 Nitric oxide controls a diverse range of pulmonary functions, such as macrophage activity, pulmonary artery vasodilation, and bronchoconstriction,5 and it has been proposed to be an important factor in developing PAH. In actuality, decreased bioavailability of NO in the lung tissue is associated with PAH, and NO inhalation causes selective vasodilation in PAH.6

Interestingly, elevated levels of an endogenous competitive inhibitor of NOS, asymmetric dimethylarginine (ADMA), has been reported in the plasma of patients with chronic thromboembolic pulmonary hypertension, idiopathic PAH, or PAH related to sickle cell disease or systemic sclerosis, indicating a strong association of circulating ADMA levels with PAH pathogenesis.7 Asymmetric dimethylarginine may therefore control pulmonary cell functions either via direct effects of gene expression and protein function, or via inhibition of NOS and secondary NO generation.7 Importantly, the lung generates a significant amount of ADMA itself, and as such may directly contribute to tissue and plasma ADMA levels, further suggesting that dysregulated ADMA metabolism in the lung may trigger, initiate, or mediate PAH.7

The methylarginines ADMA and monomethyl arginine are endogenously produced amino acids that inhibit NOS and are derived from the proteolysis of methylated arginine residues on various proteins by protein methyltransferases.7,8 These methylarginines are subsequently hydrolyzed and degraded by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) into citrulline and methylamines.7,8 DDAH is widely distributed throughout the body, and there are 2 isoforms of DDAH: DDAH-1 and DDAH-2.9 Dimethylarginine dimethylaminohydrolase-1 is expressed in tissues expressing neuronal NOS, and DDAH-2 predominates in tissues containing eNOS. Dimethylarginine dimethylaminohydrolase is expressed in pulmonary vessels, and increased plasma level of ADMA is accompanied by decreased DDAH (either DDAH-1 or DDAH-2) activity in animal models of PAH. Most importantly, lung tissues obtained from idiopathic PAH have demonstrated an impaired expression of DDAH-2, but...
not DDAH-1. Thus, the ADMA/DDAH balance could be a critical regulator of vascular endothelial function and vascular remodeling in conditions where NO/ADMA balance play an important role in disease pathogenesis and prognosis.

Because PAH is associated with a decreased bioavailability of NO, targeting the NO pathway is considered a potential therapy. The potent vasodilator and antiproliferative activity of NO is mediated via its downstream second messenger signaling molecule cGMP, which is also regulated by enzyme PDEs. Enhanced activities of PDEs, which hydrolyze NO and prostaglandin-induced cGMP and cAMP, were observed in experimental conditions of PAH. Interestingly, PDE-5 is abundantly expressed in lung tissue; thus PDE-5 inhibitors are a mainstay of PAH therapy. The PDEs have different tissue distributions and substrate affinities, and the mechanism of action of PDE inhibitors is dependent on a functional upstream signaling cascade, ie, an intact NO-soluble guanylyl cyclase (sGC)-cGMP pathway. Because this signaling pathway is impaired on many levels in pulmonary and systemic vascular disorders, it could be advantageous to identify the potential modulator and tackle this important vasodilative/antiproliferative pathway.

In this issue of Circulation, Pullamsetti et al demonstrate that therapeutic inhalation of combined PDE-3/4 inhibitor tolafentrine reduced monocrotaline-induced PAH in a rat model. Phosphodiesterase 3 and 4 isoforms are the essential players coregulating cAMP catabolism in many organs, including the lungs, and are reported to be upregulated in experimental PAH models. Compared with sham nebulization, the authors showed that 4 weeks of tolafentrine therapy improved hemodynamic values and reduced vascular remodeling and endothelial degeneration in monocrotaline-induced PAH rats.

Tolafentrine has been shown to amplify the vasodilatory effect of inhaled prostacyclin analog iloprost; however, its mechanisms of action and long-term effects on pulmonary vascular remodeling in PAH are unknown. The major step forward in the present study is the attempt to understand the mechanisms of this therapeutic intervention in the vascular remodeling of PAH. The authors emphasized that the potential beneficial role of tolafentrine lies in modulating the ADMA-DDAH-NO axis. They showed that tolafentrine treatment increased DDAH2 mRNA levels, restored DDAH expression and activity in lung tissue, decreased plasma ADMA levels, increased NO synthesis with increased NOX and cGMP levels, increased pulmonary vascular density, and decreased the number of apoptotic pulmonary endothelial cells. Interestingly, tolafentrine treatment selectively reduced total cAMP-specific PDE activity only in the lung tissue, compared with other organs. Overall, these findings expand the authors’ earlier reports and add further support for the concept of cAMP-PDE-3/4 inhibition as a new therapy in PAH.

An appealing mechanism by which to better realize the benefits of targeted therapy on the ADMA-DDAH-NO axis may be the enhancement of protein expression or the modulation of the posttranslational modification and activity of eNOS in pulmonary artery and lung tissue of PAH rats with and without tolafentrine treatment. This was not explored in detail by Pullamsetti et al in the monocrotaline-PAH model except in human umbilical vein endothelial cells. It is noteworthy that elevated levels of endogenous methylarginines have been demonstrated in a pathological model of vascular injury and restenosis where the cellular methylarginines reached the concentrations sufficient to inhibit NOS function and endothelium-dependent vasodilation in rats. Interestingly, it has also been shown that elevated levels of methylarginines can induce NO uncoupling with enhanced NOS-mediated reactive oxygen species formation.

With regard to posttranslational modification of eNOS, it has recently been shown that oxidative stress triggers S-glutathionylation, which causes eNOS uncoupling, switching eNOS from its classical NO synthase function to that of an NADPH-dependent oxidase generating superoxide that is not inhibited by NOS inhibitors. S-glutathionylation of eNOS is increased in hypertensive vessels, resulting in endothelial dysfunction. Although S-glutathionylation of eNOS is proposed to be a pivotal switch providing redox regulation of cellular signaling, endothelial function, and vascular tone, its role in the etiology of PAH is unknown.

Dimethylarginine dimethylaminohydrolase activity in endothelial cells is decreased under oxidative stress, and DDAH also is subject to extensive posttranscriptional inhibition by an NADPH-dependent oxidase or by homocysteine. It is reported that nitrosylation of the sulfhydryl group of cysteine-249 reversibly inhibits DDAH activity, and that oxidative inhibition of DDAH activity causes excessive accumulation of ADMA and pathological inhibition of NOS. This dysregulation of DDAH is thought to impair NO synthesis and NO-mediated angiogenesis. Furthermore, protein methyltransferases expression is also found to be upregulated in mice exposed to chronic hypoxia, resulting in increased ADMA tissue levels and a decreased L-Arginine/ADMA ratio, thereby supporting an important role of protein-methyltransferases-mediated ADMA generation in hypoxia-induced PAH. Although it is beyond the scope of this editorial to review all possible cascades and associated mechanisms of ADMA-DDAH-NO signaling pathways, the close interaction between the NOS/DDAH pathways and those that generate or mobilize reactive oxygen species may underlie the finding that both an elevation of ADMA and an elevation of reactive oxygen species are implicated in vascular and organ dysfunction.

cGMP-dependent protein kinase, or protein kinase G, is one of the major intracellular receptors for cGMP. Activation of protein kinase G phosphorylates several intracellular proteins that in turn regulate many important physiological functions such as control of vascular tone, cell differentiation and proliferation, and platelet aggregation. Phosphodiesterase -5 inhibitor sildenafil is reported to activate protein-kinase-G-dependent novel cytoprotective signaling cascades with enhanced Bel-2/Bax ratio, phosphorylation of Akt, ERK1/2, and glycogen synthase kinase 3β. Activated Akt and protein kinase A can also phosphorylate eNOS and activate the eNOS-NO signaling pathway. These pathways are not well-addressed in relation to PDE-5 inhibitors in PAH therapy, but they could play a significant role in the pharmacological outcome of cAMP-PDE-3/4 inhibition.
Pullamsetti et al. have shown a potential direction toward targeted drug-based treatment of PAH. We must now define how—and in which conditions—it may be useful. Pulmonary arterial hypertension therapies are distinguished by 3 approaches: (1) therapies that improve endothelial function, promote angiogenesis, and induce vasodilation, (2) antiproliferative and proapoptotic therapies toward pulmonary vascular smooth muscle cells, and (3) antiinflammatory and antioxidant therapies. Although many of these therapies, including the study of Pullamsetti et al., have demonstrated efficacy in monocrotaline-induced PAH in animals, there is a need to investigate other animal models of PAH before we recommend any therapy for human use, because PAH can result from a wide variety of causes. Better pharmacological therapies are very much needed for PAH, and Pullamsetti et al. have identified a new therapeutic approach of combined cAMP-PDE-3/4 inhibition. Further studies will be needed to assess the promise and efficacy of this novel approach in PAH.

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