JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 7, 2024

EDITORIAL COMMENT

Selective Inhibition of Peripheral Serotonin Synthesis in Pulmonary Hypertension

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Pulmonary hypertension (PH) is a pulmonary vasculopathy characterized by progressive proliferative remodeling of the small pulmonary arteries. With a prevalence of approximately 1% of the global population, PH is a rare disease, but it continues to be a major cause of cardiovascular illness and death. PH is a heterogenous group of disease entities that is classified, based on etiology and pathophysiology, into 5 subgroups.[1] PH is diagnosed if the mean pulmonary arterial pressure is >20 mm Hg at rest, highlighting the hemodynamic definition of the disease,[2] and is frequently accompanied by right ventricular (RV) hypertrophy, ultimately progressing to (right) heart failure and death. The pathologic substrate underlying the increase in pulmonary arterial pressure is the uncontrolled growth, proliferation, and remodeling of pulmonary vascular cells leading to intimal and media thickening and, as the disease progresses, vessel lumen obliteration. Although prior research has revealed both hereditary and acquired causes of PH, the pathophysiology is multifactorial and complex, and is far from being fully understood.

Despite significant scientific discoveries in the past decades, PH remains an incurable disease. Current treatment strategies and available drugs primarily target the vasoactive mediators and mitogenic signaling pathways underlying the vasoconstriction and uncontrolled proliferative remodeling of the pulmonary vascular cells, which is the nitric oxide–cyclic guanosine monophosphate and the endothelin-pathway, or act as prostacyclin agonists.[2] More recently, drugs such as sotatercept, a recombinant activin receptor type IIa IgG-Fc fusion protein that acts as a ligand trap for members of the transforming growth factor-β superfamily, were evaluated; they were shown to be highly efficacious in improving the functional exercise capacity in patients with PH, on top of the standard of care background therapy.[3]

Targeting serotonin synthesis or signaling has been considered an additional option for several years for the treatment of patients with PH. Serotonin (also known as 5-hydroxytryptamine) is a neurotransmitter and regulates numerous physiological processes in the brain. It has become known as a “feel-good hormone” based on its effects on mood, sleep, and other activities. However, serotonin also acts as an important signaling molecule in the periphery. Although the majority of peripheral serotonin is produced in the gastrointestinal tract to control gut motility, neuroendocrine and endothelial cells in the lung, among others, also synthetize this neurotransmitter. In fact, serotonin, together with endothelin-1, is one of the most potent vasoconstrictors in the pulmonary circulation and also a strong mitogen for pulmonary endothelial cells and smooth muscle cells (SMCs).

The first hints that serotonergic signaling may play a role in the pathophysiology of PH originated in the 1960s and the development of pulmonary fibrosis in individuals taking appetite suppressants such as fenfluramine, aminorex, and others, which led to the “serotonin hypothesis of pulmonary hypertension.”[4,5] Designed to control appetite and food intake by increasing serotonin levels in the central nervous system, these pharmaceutical agents also

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors’ institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2024.05.009

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 9, NO. 7, 2024
JULY 2024:903–905

increased serotonin levels in the periphery, including the lungs, with detrimental consequences. Persistent PH of the newborn was seen in association with the intake of serotonin reuptake inhibitors to treat depression or other psychiatric conditions in late pregnancy. Serotonin levels are elevated in patients with PH, possibly due to increased serotonin production by pulmonary neuroendocrine and endothelial cells or release from the dense granules of activated platelets; an impaired ability of lung endothelial cells to metabolize serotonin also may play a role.

Although all of this information has been known for some time, one wonders why serotonin antagonists have not made it into the clinic. In fact, inhibiting serotonin receptors (using terguride or ketanserin) or serotonin transporters (using citalopram and fluoxetine) successfully reduced the exaggerated SMC proliferation in vitro and improved PH severity in the monocrotaline and hypoxia rat models.[6,7] However, these inhibitors are not specific for serotonin, and clinical studies failed to reach the predetermined endpoint. REVEAL (Registry to Evaluate Early and Long-term PH Disease Management) even showed that the intake of selective serotonin reuptake inhibitors was associated with an increased mortality and greater risk of clinical worsening.[8] With these disappointing findings, the interest in alternative, more selective ways to target the serotonin pathway in PH has risen.

In this issue of *JACC: Basic to Translational Science*, Legchenko et al[9] test the effectiveness of a novel, highly specific, and orally available inhibitor (called TPT-001) targeting the peripheral isoform of the serotonin-synthesizing enzyme tryptophan hydroxylase (TPH). The hydroxylation of tryptophan, the first and rate-limiting step of the serotonin biosynthesis, is catalyzed by the enzyme TPH, of which 2 isoenzymes are known to exist in most vertebrates: TPH-2 is predominantly expressed in neuronal cells, and TPH-1 is the primary isoform in peripheral cells and is expressed at increased levels in lungs and pulmonary endothelial cells from patients with PH. Thus, the TPH-1 isoenzyme represents an ideal target to overcome the serious side effects of interfering with the serotonin release and uptake mechanism mentioned earlier. TPT-001 is unable to pass the blood-brain barrier, and it therefore selectively reduces serotonin synthesis in the periphery while preserving serotonin levels in the central nervous system.

To determine its effectiveness in treating PH in vivo, Legchenko et al[9] used the Sugen/hypoxia (SuHx) rat model. In this model, PH is induced by subjecting the animals to hypoxia and injecting Sugen (SU5416), a vascular endothelial growth factor receptor inhibitor. The SuHx model is not only a major model for the study of PH, but it may have been particularly suited to address the role of inhibiting serotonergic signaling given previous observations that serotonin receptors and transporters are strongly up-regulated under hypoxic conditions.[10,11] Animals were treated with TPH-001 at a time point when PH was already present but right heart failure had not (yet) developed. Legchenko et al[9] show that 5 weeks of treatment of rats with TPT-001 is able to reverse the disease, as shown by reduced right ventricular pressure, hypertrophy, and dilation using transthoracic echocardiography and invasive cardiac hemodynamic measurements. Histological analysis revealed that TPH-1 inhibitor treatment also reduced the presence of proliferating cells in the lung and perivascular infiltration with macrophages and T cells, a frequent, albeit uncharacteristic, feature of PH. It remains unclear whether this observation is a direct consequence of the inhibitor (eg, by targeting TPH-1 receptors expressed on immune cells). Of note, TPT-001 was used as monotherapy, and the beneficial effects can therefore be attributed solely to this treatment. Previous studies have compared TPH-1 inhibitor monotherapy vs combinatorial therapy using conventional PH drug classes used in the clinic.[12]

Inhibition of the peripheral serotonin synthesis by targeting TPH1 to treat PH has been tested in the past. For example, rodatristat ethyl, a prodrug of the peripheral TPH1 inhibitor rodatristat, dose dependently decreased serum, gut, and lung serotonin levels and significantly reduced pulmonary vessel wall thickening in 2 preclinical rat models of PH; significant effects on pulmonary hemodynamics were only seen at higher dosages.[12] In the ELEVATE 2 (A Study of Rodatristat Ethyl in Patients With Pulmonary Arterial Hypertension-2; NCT04712669) study, a phase 2b, double-blind, multicenter clinical trial, patients with PH were randomized to the rodatristat group vs the placebo group. Recruitment is complete; the results remain to be published. What distinguishes the novel TPH-1 inhibitor from previous ones is that the xanthine-benzimidazole derivative spans all active binding sites important for catalysis.

Another novel aspect of this study is the unbiased analysis of overall changes in gene expression patterns in SuHx rat lungs in response to lung disease induction as well as treatment.[9] Whole lung messenger RNA sequencing in SuHx rats revealed gene expression patterns related to SMC proliferation and vasodilation but also reactive oxygen species and

inflammation, and some of the differentially expressed genes have not been previously related to the pathophysiology of the disease. Nevertheless, the exact mechanisms of how TPH-1 promotes pulmonary remodeling and PH and the pathways involved in PH reversal remain to be elucidated. The present study is a big first step in favor of this endeavor.

Although the findings are promising and show the superior potency of TPH-001 to peripheral tryptophan hydroxylase inhibitors that are already clinically approved for the treatment of serotonin-related diseases or symptoms,[9] the route to future application in patients with PH is still a long way ahead; in addition, side effects have to be excluded and long-term consequences determined. For example, TPH-1 deficiency in mice was shown to exacerbate renal injury and fibrosis by activating nuclear factor κB.[13] The cause of these discrepancies is unclear at the moment, but the beneficial effects of inhibiting serotonin production may be organ specific. It should also be noted that only male rats were examined in this study[9] and in previous studies, whereas activation of the serotonin system is observed particulary in female patients. An opportunity to study the importance of serotonin for the known effects of sex in the pathophysiology of PH may have been missed.

In summary, the findings of the study by Legchenko et al[9] using a novel class of selective, highly potent peripheral tryptophan hydroxylase inhibitors may rekindle interest in serotonin, a long-known player in the pathophysiology of PH and more than just a "feel-good hormone." Its use as a novel therapeutic approach to specifically inhibit the detrimental activities of the neurotransmitter in the lung, including its activities as vasoconstrictor and mitogen, but also beyond need investigation.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This study was supported by research funding from the German Research Foundation (Deutsche Forschungsgemeinschaft; SCHA 808/9-2 and 15-1) and the Collaborative Research Center (1531, project A09; project number 456687919). Dr Schäfer is Principal Investigator of the German Center for Cardiovascular Research (DZHK), partner site Rhine-Main. Dr Bikou is a DZHK Scientist, partner site Munich Heart Alliance.

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REFERENCES

1. Ruopp NF, Cockrill BA. Diagnosis and treatment of pulmonary arterial hypertension: a review. *JAMA*. 2022;327(14):1379–1391.

2. Humbert M, Kovacs G, Hoeper MM, et al. 2022 ESC/ERS guidelines for the diagnosis and treatment of pulmonary hypertension. *Eur Heart J*. 2022;43(38):3618–3731.

3. Hoeper MM, Badesch DB, Ghofrani HA, et al. Phase 3 trial of sotatercept for treatment of pulmonary arterial hypertension. *N Engl J Med*. 2023;388(16):1478–1490.

4. Simonneau G, Fartoukh M, Sitbon O, Humbert M, Jagot JL, Hervé P. Primary pulmonary hypertension associated with the use of fenfluramine derivatives. *Chest*. 1998;114(3 Suppl):195s–199s.

5. Abenhaim L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, et al. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N Engl J Med*. 1996;335(9):609–616.

6. Marcos E, Adnot S, Pham MH, et al. Serotonin transporter inhibitors protect against hypoxic pulmonary hypertension. *Am J Respir Crit Care Med*. 2003;168(4):487–493.

7. Dumitrascu R, Kulcke C, Königshoff M, et al. Terguride ameliorates monocrotaline-induced pulmonary hypertension in rats. *Eur Respir J*. 2011;37(5):1104–1118.

8. Sadoughi A, Roberts KE, Preston IR, et al. Use of selective serotonin reuptake inhibitors and outcomes in pulmonary arterial hypertension. *Chest*. 2013;144(2):531–541.

9. Legchenko E, Chouvarine P, Qadri F, et al. Novel tryptophan hydroxylase inhibitor TPT-001 reverses PAH, vascular remodeling, and proliferative-proinflammatory gene expression. *JACC Basic Transl Sci*. 2024;9(7):890–902.

10. Eddahibi S, Fabre V, Boni C, et al. Induction of serotonin transporter by hypoxia in pulmonary vascular smooth muscle cells. Relationship with the mitogenic action of serotonin. *Circ Res*. 1999;84(3):329–336.

11. Bikou O, Sassi Y. The sugen/hypoxia rat model for pulmonary hypertension and right heart failure. *Methods Mol Biol*. 2024;2803:163–172.

12. Aiello RJ, Bourassa PA, Zhang Q, et al. Tryptophan hydroxylase 1 inhibition impacts pulmonary vascular remodeling in two rat models of pulmonary hypertension. *J Pharmacol Exp Ther*. 2017;360(2):267–279.

13. Chen D-Q, Cao G, Chen H, et al. Identification of serum metabolites associating with chronic kidney disease progression and anti-fibrotic effect of 5-methoxytryptophan. *Nat Commun*. 2019;10(1):1476.

KEY WORDS hypoxia, pulmonary hypertension, rat model, right heart failure, serotonin, Sugen, therapy, tryptophan hydroxylase 1