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Sex Hormones and Vascular Protection in Pulmonary Arterial Hypertension

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estrogen; endothelium; nitric oxide; vascular smooth muscle; calcium; blood pressure

Pulmonary Arterial Hypertension (PAH) is a serious disease and a major public health problem with approximately 1000 new patients diagnosed every year in the United States.[1, 2] PAH is a progressive disease involving impaired pulmonary vascular structure and function and is ultimately lethal due to right ventricular failure. Recent insights into the pathogenesis of PAH have lead to more promising therapeutic approaches and improved outcomes; however, the mortality rates associated with PAH remain unacceptably high.[1, 3, 4]

PAH affects all age groups and both genders; however, there is a striking preponderance of female PAH patients which remains unexplained.[5] PAH can be idiopathic, familial, or associated with other cardiovascular disorders, but the underlying pathology and pathophysiology are shared by all forms of the disease. PAH is characterized by progressive fibro-proliferative remodeling of the pulmonary arterioles, various degrees of pulmonary vasoconstriction and inflammation, thrombosis, and right ventricular hypertrophy and failure. The mainstay of current pharmacologic therapies is pulmonary vasodilation, although only a small percentage of patients have demonstrable pulmonary vasoconstriction when they undergo cardiac catheterization.[6] Despite the lack of a demonstrable vasoconstrictor component, many PAH patients improve with long-term vasodilator therapy, and it is believed that pulmonary vascular remodeling could also be ameliorated as a result of vasodilator therapies. Vasoconstrictors such as angiotensin II (AngII) and endothelin-1 (ET-1) are potent stimulators of vascular smooth muscle (VSM) growth and proliferation. While many vasodilator mediators also have anti-proliferative effects on VSM, there is no definitive evidence that pulmonary vascular remodeling in humans with PAH is reversible. In addition, current vasodilator therapies are not universally successful in altering PAH progression and increasing survival. Therefore, novel approaches that directly target pulmonary vessel wall pathology are needed in order to reverse the established pulmonary vascular pathology in PAH patients.

Pathophysiological Mechanisms of PAH

Studies in human PAH and experimental models of the disease have suggested the involvement of several molecular and signaling pathways in the development and progression of PAH.[7–20] Mutations in bone morphogenetic protein receptor type II (BMPR2) and transforming

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growth factor beta (TGF-β) receptor have been associated with familial primary PAH.[8] Decreased release or activity of endothelium-derived nitric oxide (NO) in the pulmonary circulation and loss of NO-cGMP relaxation through degradation of cGMP via phosphodiesterase 1 (PDE1) are major factors in the pathogenesis of PAH (Figure).[7, 12] Several other pathways have been implicated in PAH pathogenesis as demonstrated by loss of function and interventional studies: these include cyclooxygenase-2 (COX-2) and prostacyclin (PGI_2),[17, 19] ET-1 [15] and platelet-derived growth factor (PDGF) signaling,[13] the Rho-kinase [14] and Notch3 signaling pathways [20] as well as heme oxygenase-1 (HO-1)/carbon monoxide (CO).[18, 21] Nevertheless, PAH appears to be a multifactorial disorder, and more than one gene or signaling pathway is likely to be involved.

As demonstrated in human studies as well as studies in experimental animals, PAH is a pan-vasculopathy characterized by endothelial dysfunction, medial hypertrophy and VSM hyperplasia, and adventitial fibrosis. Using the monochrotaline (MCT) and hypoxic rat models of PAH, we and others have shown reduced pulmonary artery contraction to vasoconstrictors, decreased endothelium-dependent NO-cGMP-mediated pulmonary artery relaxation, and decreased pulmonary artery responsiveness to endogenous and exogenous nitrovasodilator.[7, 22–25] The changes in pulmonary artery function are associated with extensive pulmonary artery thickening and remodeling, and increased pulmonary VSM cell growth and proliferation (Figure).[26–29] These observations have made the MCT and hypoxic rat models the most commonly used animal models to investigate the pathophysiology of PAH and to test the effects of potential therapies of the disease. In addition to the significant pulmonary artery remodeling, perivascular inflammation and transdifferentiation of circulating and resident progenitor cells are thought to contribute to the pathogenesis of PAH via mechanisms that are incompletely understood.[30–33]

Common Therapies of PAH

The characterization of the molecular mechanisms underlying PAH has been critical to identifying novel targets for therapeutic intervention. However, as novel targets are identified, multiple obstacles to clinical application have to be overcome since bioavailability, selectivity, and potential toxicity of novel therapies need to be carefully evaluated in clinical trials. As a result, despite the identification of multiple potential new targets for intervention, only three classes of therapies are currently in use for PAH. These include PDE-5 inhibitors, PGI_2 analogs, and endothelin receptor A (ET_AR) antagonists. These therapies target molecular pathways that are known to be dysregulated in the setting of PAH. PDE inhibitors such as sildenafil prevent the breakdown of cGMP and consequently enhance the NO-cGMP pulmonary arterial relaxation pathway. PGI_2 analogs such as iloprost stimulate the PGI_2 receptors and enhance the PGI_2-cAMP relaxation pathway. Non-specific ET-1 receptor antagonists such as bosentan and specific ET_AR antagonists like sitaxentan and ambrisentan block ET-1 induced signaling and its effects on both VSM contraction and cell growth. To enhance their effectiveness, these therapeutic approaches are often used in combination, although the optimal combination therapy is still under investigation.[34, 35] Whether used separately or combined, the goal of these approaches is to restore the balance between the NO-cGMP and PGI_2-cAMP vasodilator and VSM anti-proliferative pathways, and the ET-1 induced vasoconstrictor and VSM proliferative pathway in the pulmonary vasculature. Ca^{2+} channel blockers may have some benefits in certain PAH patients.[36, 37] Also, ample experimental evidence supports that more specific anti-proliferative, pro-apoptotic, immuno-modulatory and cell based therapies could be effective in PAH,[13, 38–44] however, translation to clinical application is lagging behind these new discoveries.

Effects of sex hormones in PAH

The preponderance of PAH in females is unexplained, yet very intriguing particularly because it is opposite of the known preponderance of systemic cardiovascular disease in males. It has become increasingly appreciated that the cardiovascular effects of sex steroids and their metabolites are far more complex than initially thought. Despite attempts to define the role of sex steroid hormones in pulmonary vascular disease, significant knowledge gaps exist in this area.[44] Most studies on the role of sex steroids in pulmonary vascular homeostasis focus on the vasodilator properties of estrogens, and possibly androgens, especially in the settings of experimental hypoxia.[45] Specifically, 17-hydroxy-estradiol has been reported to have multiple effects on the endothelial production of NO and PGI_2 which in turn lead to endothelium-dependent vasodilation. In addition, 17-hydroxy-estradiol has endothelium-independent vasodilatory effects by activating voltage-activated potassium channels in VSM cells. Similarly, progesterone and testosterone have been reported to reduce vascular tone by blocking both voltage-gated and receptor-operated Ca^{2+} channels in VSM cells.[45] Recently, 2-methoxy-estradiol (2-ME) has been recognized as a biologically active metabolite of estradiol with estrogen receptor-independent anti-proliferative properties [46] and its therapeutic potential in cardiovascular and pulmonary vascular diseases warrant further investigation.

In this issue of the *Journal of Cardiovascular Pharmacology,* Tofovic and co-workers present experimental evidence supporting that 2-ME treatment ameliorates MCT-induced PAH not only in adult females, but also in adult male rats. 2-ME was as efficacious as the PDE inhibitor sildenafil and the ET-1 receptor antagonist bosentan in ameliorating MCT-induced PAH in both male and female rats. The authors also demonstrate that the combination of 2-ME with either sildenafil or bosentan confers additional protection and improves survival in MCT-treated rats (Figure). Importantly, these pharmacological interventions were initiated twelve days after administration of MCT and the establishment of PAH and pulmonary vascular remodeling thus supporting effective reversal of established disease. The authors propose that the mechanism of protection involves anti-proliferative and anti-inflammatory effects of 2-ME in the pulmonary vasculature and the lung, respectively.

Future Directions

The study by Tofovic and colleagues opens an important area for investigation with regard to the sex differences in the incidence of PAH and the role of sex hormones in the pathogenesis and management of PAH. An important question is whether the increase in the incidence of PAH in females is related to estrogen. This will be difficult to reconcile with the apparent protective effects of 2-ME in the MCT-treated model of PAH. One possibility is that the effects of estradiol on the pulmonary vasculature are different from those of estrogen metabolites. In this respect, it is important to compare the effects of 2-ME on pulmonary vessels with those of estrogen, specific estrogen receptor modulators, and other estrogen metabolites. It will also be important to study the differential effects of estrogens as compared to androgens on the pulmonary vasculature.

The specific mechanisms underlying the effects of estrogen and its metabolites on pulmonary vascular structure and function also need to be further examined. Estrogen-induced endothelium-dependent vasodilator effects on NO, PGI_2 and endothelium-derived hyperpolarizing factor (EDHF) have been described in blood vessels of the systemic circulation.[47] Additional inhibitory effects of estrogen on VSM cell contraction and growth have been suggested.[47] Studies have suggested inhibitory effects of estrogen on the Ca^{2+}-dependent mechanisms of VSM contraction.[48] Other studies have suggested an inhibitory effect of estrogen on the expression/activity of protein kinase C and Ca^{2+}-sensitization pathways of VSM contraction (Figure).[49] In this respect, studies have suggested a role for Rho-

kinase as Ca^{2+}-sensitization pathway of VSM contraction as well as in VSM cell growth and proliferation.[50] Given the beneficial effects of Rho-kinase inhibitors in experimental PAH,[14] it would be important to test whether estrogen or its metabolites function by inhibiting Rho-kinase. Estrogen and its metabolites may also affect the expression/activity of matrix metalloproteinases and the composition of the extracellular matrix,[51] and such effects could also improve pulmonary arterial remodeling in PAH.

Finally, while studying the effects of 2-ME in MCT-treated rat model highlights potential pulmonary vascular protective effects in PAH, it would be important to study the effects of estrogen and its metabolites in other animal models such as the hypoxia-induced model of PAH. Careful evaluation of the findings in experimental animal models could spearhead studies in human and clinical trials to determine the effects of sex hormones on the course of PAH.

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List of abbreviations

cGMP	cyclic guanosine monophosphate
COX-2	cyclooxygenase-2
ET-1	endothelin-1
ET_AR	endothelin receptor A
MCT	monochrotaline
2-ME	2-methoxy-estradiol
NO	nitric oxide
PAH	pulmonary arterial hypertension
PGI_2	prostacyclin
PDE	phosphodiesterase
VSM	vascular smooth muscle

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Figure.
Pathophysiological mechanisms and molecular targets in MCT-treated and hypoxia-induced model of PAH. The PAH-associated endothelial cell dysfunction and decreased pulmonary artery relaxation, and the VSM dysfunction and increased pulmonary artery constriction and remodeling are reduced by treatment with the PDE inhibitor sildenafil, or the ET-1 receptor antagonist bosentan. 2-ME enhances the stimulatory effects of sildenafil on pulmonary artery relaxation, and the inhibitory effects of bosentan on vasoconstriction and remodeling.