Cyp2c44-mediated decrease of 15-HETE exacerbates pulmonary hypertension

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Abstract

Pulmonary hypertension (PH) is a progressive disease characterized by elevated pulmonary arterial pressure and pulmonary vascular resistance. According to latest estimations, a PH incidence accounts for 1% of total global population (up to 5 million people) and the median survival rate for patients diagnosed with PH is predicted at 2.8 years. Emerging evidence suggests an important role for cytochrome P450 in the development and progression of PH, as a newer member of cytochrome family Cyp2c44 has been recently implicated in mediating hypoxia-induced pulmonary artery remodeling. Recent studies show that Cyp2c44 effects on the pathophysiology of PH are driven by its ability to produce 15-hydroxyeicosatetraenoic acid (15-HETE). In this article, we discuss new mechanisms by which Cyp2c44 and 15-HETE contribute to the progression of PH.
Pulmonary hypertension (PH) is a progressive disease characterized by elevated pulmonary arterial pressure and pulmonary vascular resistance (19). According to latest estimations, a PH incidence accounts for 1% of total global population (up to 5 million people), and its rate increases up to 10% in individuals above 65 years of age (24). The median survival rate for patients diagnosed with PH is predicted at 2.8 years (8).

PH develops as a result of vasoconstriction and remodeling in the intra-alveolar pulmonary arteries and leads to progressive right ventricular hypertrophy and cardiac failure (1, 11, 27, 29, 31, 34, 37). Pulmonary vasoconstriction is thought to be an early component of PH and has been primarily attributed to vascular endothelial dysfunction that leads to impaired production of vasodilators such as nitric oxide and prostacyclin, and overexpression of vasoconstrictors such as endothelin-1 (25). According to recently published data, there are currently 10 drugs from five different substance classes available and licensed for the treatment of PH, including endothelin receptor antagonists, phosphodiesterase-5 inhibitors, stimulators of soluble guanylate cyclase, and arachidonic acid pathway targeted drugs such as prostacyclin analogues and prostacyclin receptor agonists (23). These medications are typically prescribed singly or in combination and their primary focus is to induce vasodilation of the pulmonary arteries. A recent randomized trial that utilized a combination of these classes of medications has reported a 40% success rate in patients with PH (18). In addition, the use of some medications such as endothelin-1 receptor antagonists remains limited due to their known hepatotoxicity and several drugs from this class have been already withdrawn from clinical practice (6). These data suggest that other options should be investigated to develop new therapeutic targets to improve the outcomes in patients with PH.

While the cyclooxygenase and lipoxygenase metabolites of arachidonic acid metabolism have been well-studied and clinically implicated in prevention of endothelium-targeted vasoconstrictor effects during PH, the cytochrome P450 (CYP) pathway of arachidonic acid metabolism remains understudied. Because CYP proteins are sensitive to changes in oxygen tension and are widely expressed in lung tissues (38), there is an increasing need to further study the effects of CYP on the progression and development of PH. For example, an intriguing recent study from the Gupte laboratory reported the importance of Cyp2c44 gene in the progression of PH and showed that Cyp2c44 gene deletion results in increased hypoxia-induced pulmonary artery remodeling and hypertension (26). The Cyp2c44 gene is broadly expressed and studies show that it may be found in kidney, liver, and adrenal glands (12, 26, 35). In original studies on the arachidonic acid metabolism proposed the primary role for CYP in generation of EETs, however, more recent studies report that Cyp2c44 in particular is capable of generating 15-hydroxyeicosatetraenoic acid (15-HETE), and that the amount of 15-HETE produced by Cyp2c44 accounts for up to 23% of total 15-HETE production (12).

In the current issue of the American Journal of Physiology-Heart and Circulatory Physiology, Hashimoto et al. (21) propose a new role for the effects of CYP450 family proteins on hematopoetic progenitor stem cells in mice. The authors showed that Cyp2c44 gene deletion leads to a decrease in 15-HETE production and to an increase in the numbers of CD133+ hematopoetic progenitor stem cells. The evidence of the relationship between PH and CD133+ progenitor cells had been accumulating for the past decade, although the reported relationship was only correlative. As such, increased numbers of circulating and tissue levels of CD133+ cells have been reported both in pre-clinical models and in patients with PH (2, 14, 28, 30, 32, 36). In addition, transplantation of bone marrow CD133+ cells from patients with pulmonary hypertension into mice resulted in angioproliferative pulmonary vascular remodeling, right ventricular failure, and death, suggesting a pathophysiological mechanism for CD133+ cells (3). In this manuscript, Hashimoto et al. provide the mechanistic link between CYP pathway of arachidonic acid metabolism showing a decrease in 15-HETE production and an increase in
accumulation of CD133+ cells in the adventitia surrounding the medial layer of the remodeled pulmonary arteries in lungs of Cyp2c44 null mice with PH. These data suggest that Cyp2c44 gene deletion and subsequent reduction in 15-HETE levels further worsens and actively participates in the remodeling processes during PH. The data produced by Hashimoto and colleagues support previously published data of the involvement of CD133+ cells in the development of plexiform lesions in the arteries of patients with PH (7, 33). These findings indicate a possible beneficial effect of 15-HETE in the remodeling of arteries during PH. However, the existing knowledge of the diverse biological actions of 15-HETE (10) indicates a necessity for future studies on the compensatory mechanisms that are activated due to Cyp2c44 gene deletion.

Hashimoto and colleagues show that 15-HETE is capable of directly affecting the CD117+ precursor of endothelial cells. In the in vitro experiments, 15-HETE significantly reduced number of CD117+ cells when incubated with bone marrow cells. Authors link the increased circulating levels of CD117+ cells and increased expression of von Willibrand factor+ cells in the lungs of Cyp2c44 mice, indicating that in addition to active remodeling of the arteries during pulmonary hypertension, 15-HETE may also have a strong effect on the formation of neointimal lesions. These data suggest that 15-HETE also plays an important role in altering the proangiogenic responses during pulmonary hypertension. In addition, the presence of CD117+ cells in the hypertrophied hearts of the Cyp2c44 null mice also indicates possible roles of 15-HETE in heart failure during PH. In 2015, the Global Burden of Disease Study reported more than 60 million heart failure patients worldwide and its predicted that up to 50% of these patients could develop PH (20). Thus, finding a pathway that plays a role both in the pathology of failing heart and PH might serve as a potentially effective therapeutic target. However, further studies to dissect specific roles of 15-HETE in the failing heart and during the development of PH are warranted.

Inflammation is associated with most forms of PH (4, 22). Mononuclear cells activate fibroblasts in the adventitia and pulmonary artery smooth muscle cells in the media leading to remodeling of the pulmonary arteries both in pre-clinical models and in patients with PH (2, 3, 9, 13-17). In the current manuscript (21), Hashimoto et al. also show that Cyp2c44 gene deletion, and subsequently a decrease in 15-HETE production, results in an increase of monocytes and macrophages both in bone marrow and circulation. Following previously published material which indicated that CD133+ cells can also give rise to all hematopoietic lineages (including erythroid, myeloid, monocyte/macrophage, and megakaryocytes) and the data that granulocyte/monocyte colony formation is increased in PH patients (3, 14), Hashimoto and colleagues suggest a new role for 15-HETE in mediation of inflammatory responses, subsequent arteriole wall thickening, and progression of PH.

Collectively, these findings suggest that Cyp2c44 pathway, particularly through regulation of production of 15-HETE, may have strong implications for the amelioration of PH pathophysiology (Figure 1). These findings also suggest that targeting Cyp2c44 pathway may be beneficial for the development of new therapeutic targets to improve the success rate of the currently available treatments for PH.

Among future studies that investigate the effects of Cyp2c44 gene and 15-HETE in the pathophysiology of PH, special focus should be paid to oxidative stress. Previous studies demonstrated that lung tissue from patients with severe PH exhibits significant oxidative stress and is associated with increased pulmonary production of several HETEs, including 12- and 15-HETE, and 5-oxo-ETE (5), suggesting the possible roles for Cyp2c44 gene signaling. It is also important to consider how different cells within the vessel wall use the Cyp2c44-dependent pathways and how genetic depletion of this pathways affects these cell types individually. And finally, it is important to further study how vascular remodeling processes (i.e., smooth muscle proliferation, synthesis and deposition of extracellular matrix
components) and inflammatory processes are controlled at a cellular level by Cyp2c44-dependent pathways.

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27. **Lakhkar A, Dhagia V, Joshi SR, Gotlinger K, Patel D, Sun D, Wolin MS, Schwartzman ML, and Gupte SA.** 20-HETE-induced mitochondrial superoxide production and inflammatory phenotype in


Figure legends

Figure 1. Schematic representation of Cyp2c44 and 15-HETE roles in pulmonary hypertension (PH). Upon activation, Cyp2c44 produces 15-HETE that accounts for ~30% of total 15-HETE production. During PH, 15-HETE is known to induce vasoconstriction, however, recent findings suggest that 15-HETE may also have beneficial effects in the pathophysiology of PH through attenuation of hematopoetic stem cells accumulation and inflammatory response. Future studies are needed to investigate the effects of 15-HETE on oxidative stress and vascular remodeling in PH.
Pulmonary hypertension

Tissue hypoxia

Arachidonic acid

CYP2C44 activation

15-HETE (~30% of total body 15-HETE)

EETs (77%)

HETEs (23%)

Cell

Blood vessels

Vasoconstriction

Exacerbates pulmonary hypertension

Heart

↓ proangiogenic CD117 cells

Bone marrow, blood, lungs

↓ hematopoietic CD34, CD117, CD133 cells

↓ CD11b monocytes

↓ F4/80 macrophages

Attenuates hematopoietic stem cells accumulation and inflammatory response, improves cardiac function

Unknown effects

Oxidative stress?

Cyp2c44 individual effects on other cell types?

Novel possible positive effects in pulmonary hypertension

Known negative effects in pulmonary hypertension

Unknown effects