Published in final edited form as:
Immunol Res. 2014 May ; 58(0): 387–393. doi:10.1007/s12026-014-8492-5.

Leukotrienes in pulmonary arterial hypertension

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Abstract

Leukotrienes (LTs) are lipid mediators derived from the 5-lipoxygenase (5-LO) pathway of arachidonic acid metabolism and are markers and mediators of pulmonary inflammation. Research over the past two decades has established that LTs modulate inflammation in pulmonary arterial hypertension (PAH). The purpose of this review was to summarize the current knowledge of LTs in the pathophysiology of PAH and to highlight a recent study that advances our understanding of how leukotriene B_4 (LTB_4) specifically contributes to pulmonary vascular remodeling. The results of these studies suggest that pharmacological inhibition of LT pathways, especially LTB_4, has high potential for the treatment of PAH.

Keywords

Leukotriene; Pulmonary arterial hypertension; Vascular remodeling; Inflammation; 5-Lipoxygenase

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Conflict of interest WT and MRN and Stanford University (OTL #S11-438) have a patent pending concerning the use of LTB_4 antagonists for the treatment of PAH.

Introduction

Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by vasoconstriction and vascular remodeling that result in increased pulmonary vascular resistance and pulmonary arterial pressure [1–3]. Advances in the understanding of the pathobiology of PAH have led to the development of a number of effective vasodilating therapies, such as endothelin receptor antagonists, phosphodiesterase type 5 inhibitors, and prostacyclin or analogs. However, even in the current treatment era, the average life expectancy of patients with PAH remains poor and is estimated to be 5–7 years after diagnosis [4]; new approaches are clearly needed. Perivascular inflammation is common in PAH and is characterized by the presence of various immune cells, including T cells, B cells, plasma cells, mast cells, dendritic cells, and macrophages, as well as inflammatory molecules, such as cytokines, chemokines, growth factors, eicosanoids, reactive oxygen, and nitrogen species [5, 6]. Recent preclinical studies demonstrate that abnormal regulatory T cell (Treg) activity exacerbates inflammation associated with pulmonary vascular injury and facilitates disease development [7, 8]. In PAH arising in conditions associated with immune dysregulation, the role of leukotrienes (LTs) appears to be an exciting new target for disease intervention that could complement conventional vasodilator therapy.

LTs are lipid mediators derived from the polyunsaturated fatty acid and arachidonic acid. Their function is in initiating and amplifying both the innate and adaptive immune responses by regulating the recruitment and activation of leukocytes in inflamed tissues [9, 10]. In this review, we will discuss the current understanding of how LTs are involved in several aspects of the pathogenesis of PAH.

Overview of LT synthesis and actions

LTs are synthesized primarily in leukocytes at the Golgi apparatus and endoplasmic reticulum (ER)/nuclear membrane. At these sites, activated phospholipase A2 (PLA_2), especially cytosolic PLA_2, hydrolyzes membrane phospholipids and liberates arachidonic acid from the membrane bilayer. 5-LO in the cytosol or nucleus is subsequently activated and translocates to the inner and outer nuclear membrane initiating the synthesis of LTs by converting arachidonic acid to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and LTA_4. This reaction requires the integral nuclear envelope protein 5-lipoxygenase-activating protein (FLAP) [11–13]. LTA_4 has a half-life of less than 3 s at physiological pH [14] and is quickly either conjugated to glutathione by LTC_4 synthase (LTC_4S) to form LTC_4 or hydrolyzed by LTA_4 hydrolase (LTA_4H) to generate LTB_4 [15–19]. Both LTC_4 and LTB_4 can be transported out of the source cell into the extracellular milieu. LTB_4 may also potentially act in the nucleus as a modulator of transcription. LTC_4 undergoes sequential peptide cleavage of the glutathione moiety to form LTD_4 or LTE_4 [20–23]. LTC_4, LTD_4, and LTE_4, as a group, are named the cysteinyl leukotrienes (CysLTs) (Fig. 1).

Different leukocytes generate different LT profiles: neutrophils synthesize exclusively LTB_4 [24, 25], eosinophils, and mast cells predominantly LTC_4 [26–30], while macrophages generate both [31–33]. The actions of LTs are mediated through a series of G-protein-coupled receptors. These cell-surface receptors are classified into three groups: receptors for

LTB_4 (BLT1, BLT2), receptors for CysLTs (CysLT1, CysLT2) [34], and a newly discovered receptor specific for LTE_4 known as CysLTE [35, 36]. The proinflammatory effects of LTB_4 in leukocytes are mainly transduced through the high-affinity receptor, BLT1, while the role of the lower-affinity BLT2 receptor remains unknown. The CysLTs are known to cause long-lived bronchoconstriction mediated by the CysLT1 receptor. This receptor recognizes ligands with the following rank order of the affinity: $LTD_4 > LTC_4 = LTE_4$. CysLT2 recognizes its ligands with following order of affinity: $LTC_4 = LTD_4 < LTE_4$ [37–40].

The role of the 5-LO/LT pathway in PAH

5-LO and FLAP expression in animal models and in humans with PAH

The correlation between 5-LO and PAH was first noted by Voelkel et al. in 1996; they found that both alveolar macrophages and vascular endothelial cells from rats exposed to chronic hypoxia had increased expression of 5-LO [41]. The lungs of chronically hypoxic rats showed increased translocation of 5-LO from the cytosol to the perinuclear membrane, indicating a higher level of 5-LO activation. Treatment with the FLAP inhibitor, MK-886, an antagonist for 5-LO and FLAP interaction, inhibited hypoxia-induced pulmonary vasoconstriction and prevented the development of chronic hypoxic pulmonary hypertension (PH) in rats (By convention, animal models of PAH are referred to as ‘PH’). Additionally, compared to controls, 5-LO knockout mice exposed to chronic hypoxia had attenuated right ventricle hypertrophy, as measured by the right ventricle (RV) over left ventricle (LV) plus septum (S) ratio (RV/LV + S). This was the first study to suggest that 5-LO is involved in the regulation of pulmonary vascular tone and plays a role in the pathogenesis of PH in chronic hypoxia rodent models.

The Denver group then went on to examine the expression of 5-LO and FLAP in the lungs of patients with PAH. They found that compared to healthy controls, patients with PAH had elevated protein levels of both 5-LO and FLAP demonstrated by immunohistochemistry and increased 5-LO mRNA levels measured by in situ hybridization [42]. 5-LO and FLAP were prominent in alveolar macrophages clustered around plexiform lesions. Additionally, in the lungs of PAH patients, the endothelial cells in plexiform and concentric lesions expressed both 5-LO and FLAP.

While 5-LO overexpression in rat lungs using a recombinant adenovirus expressing 5-LO (Ad5-LO) did not cause PH in normal rats, it markedly accelerated the progression of PH in rats treated with monocrotaline (MCT) [43]. An increase in pulmonary artery pressure occurred earlier in the rats treated with MCT + Ad5-LO (7–10 days) compared with those treated with control vector, or MCT alone (15–18 days). Lung tissue histological sections from MCT + Ad5-LO rats exhibited severe inflammation and pulmonary vascular muscularization. Treatment of 5-LO inhibitors, zileuton or MK-886, prevented either MCT- or MCT + Ad5-LO induced PH. These data suggested that 5-LO plays a critical role in the progression of PH in the setting of pulmonary vascular endothelial cell dysfunction [43].

BMPR2 and 5-LO in PH

Heterozygous germline mutations in the bone morphogenetic protein type 2 receptor (BMPR2) account for approximately 80 % of patients with familial PAH [44, 45]. The disease is transmitted in an autosomal dominant fashion, but has only a 20 % penetrance, which suggests that additional triggers are needed to develop the disease. Additionally, it is estimated that 15–40 % of patients with idiopathic PAH may also have mutations in BMPR2 [45–47]. The identification of this genetic mutation in PAH has provided a focus for studying the complex pathobiology of this disease.

Under unstressed conditions, *BMPR2*$^{+/-}$ mice were found to have the same life span, right ventricular systolic pressure (RVSP), and lung histology as those of wild-type mice. However, intratracheal instillation of Ad5-LO significantly increased the RVSP of *BMPR2*$^{+/-}$ mice compared to the wild-type mice. Also, modest but significant muscularization of distal pulmonary arterioles was discovered 4 weeks after Ad5-LO treatment [48]. This study showed that under the inflammatory insult triggered by 5-LO, *BMPR2*$^{+/-}$ mice were more susceptible to an increase in RVSP and vascular remodeling than wild-type mice, suggesting a critical role for 5-LO in BMPR2 haploinsufficiency and the development of PAH.

LTB_4 in PAH

Several prior observations suggested that LTB_4 may contribute to the pathogenesis of PAH. First, LTB_4 has long been recognized as a chemoattractant for many inflammatory cell types observed in PAH, such as T and B lymphocytes, macrophages, and neutrophils. Next, it is known that the phosphorylation of 5-LO (on the Ser^{271} residue by p38 MAPK) significantly increases its enzyme activity in vitro [49, 50] and, thus, facilitates 5-LO nuclear retention which favors LTB_4 biosynthesis [51, 52]. Indeed, increased LTB_4 and myeloperoxidase (MPO) level was observed within 3 days after MCT injection in rats. Administration of ONO4057, a BLT1 antagonist, reduced right ventricular hypertrophy induced by MCT and prevented these animals from developing PH [53].

Recently, our group showed that in both the SU5416 (SU; a VEGFR2 inhibitor)-treated athymic rat model of severe PH associated with an absence of T cell-mediated regulation and in the lungs of PAH patients, the expression of biosynthetic enzymes for LTB_4 production, specifically phospho-Ser^{271} 5-LO (p5-LO) and LTA_4H, were elevated compared to controls [54]. p5-LO and LTA_4H expression were prominent in macrophages around partially or fully occluded small-tomid-sized pulmonary arterioles. Upregulation of these enzymes correlated with high levels of LTB_4 in the lung bronchoalveoloar fluid as in the systemic circulation. A detailed morphometric assessment of these macrophages demonstrated that macrophages closest to diseased arterioles had the highest expression of p5-LO, suggesting that LTB_4-producing cells were in close proximity to the site of disease, possibly playing a role in the observed vascular remodeling.

Because pulmonary arterial endothelial cell (PAEC) injury is regarded as an important early event in PH pathogenesis, we established a macrophage-PAEC co-culture system to determine whether macrophages, specifically p5-LO positive, LTB_4-producing

macrophages, could injure adjacent endothelial cells. We found that macrophages isolated from the lungs of SU-treated athymic rats with PH induced significant endothelial cell apoptosis. Further, macrophages from healthy rats that were transfected with S271E 5-LO, a plasmid that produces a 5-LO phosphorylation mimic mutant, caused marked PAEC death to a similar degree. Furthermore, LTB_4 cultured with PAEC alone demonstrated a capacity to injure the cells in a dose-dependent fashion. Blocking BLT1, the major receptor for LTB_4 on endothelial cells, prevented LTB_4-mediated apoptosis [54].

To explain our findings of LTB_4-induced PAEC apoptosis, we hypothesized that LTB_4 may be inhibiting a signaling cascade vital to endothelial cell survival, such as the sphingosine 1 phosphate (S1P)—endothelial nitric oxide synthase (eNOS) pathway. S1P is a class of vasoprotective signaling lipids produced by the phosphorylation of sphingosine by sphingosine kinase 1 (Sphk1) and downstream, and activates eNOS [55, 56]. Recent studies of S1P have revealed that this bioactive sphingolipid decreases vascular permeability and promotes endothelial survival through eNOS and prostacyclin [57–59]. In this recent study [54], we showed that LTB_4 induced PAEC apoptosis by inhibiting the expression and activation of both Sphk1 and eNOS.

We further demonstrated that LTB_4 induced the proliferation and hypertrophy of human pulmonary arterioles smooth muscle cells (PASMC) in a concentration- and BLT1-dependent manner [54]. These results are consistent with the established role of LTB_4 on other types of vascular smooth muscle cells. For example, LTB_4 has been strongly implicated in the process of atherogenesis, and BLT1 expression is increased in atherosclerotic plaques and on smooth muscle cells under inflammatory stress [60]. Thus, LTB_4, liberated by activated macrophages, was produced in sufficient concentrations (as gauged by the bronchoalveolar fluid levels) to induce both PAEC apoptosis as well as PASMC proliferation, two hallmark pathologic events in PAH vasculopathy.

Because LTB_4-secreting macrophages were contiguous with diseased arterioles, we hypothesized that inhibiting LTB_4 signaling might be an effective treatment for PH. Bestatin [(2S, 3R)-3-amino-2-hydroxy-4-phenylbutanoyl-L-leucine] is a well-tolerated LTA_4H inhibitor that blocks LTB_4 formation [61, 62]. In our rat models, bestatin treatment started as late as 3 weeks after SU administration (when PH is severe and animals are near death from their cardiopulmonary disease), subsequently reduced serum LTB_4 levels, prevented PAEC injury, restored Sphk1-eNOS signaling, and rescued the animals from death. Since bestatin is known to exert pharmacological actions other than LTA_4H inhibition [63, 64], we also tested a different LTA_4H inhibitor (JNJ-26993135) [65, 66] and a BLT1 antagonist (LY293111) [67, 68]. Treatment with both of these agents also reversed PH and prevented PH-related death. While bestatin was also effective with reversing MCT-induced PH, it was ineffective in the SU-chronic hypoxia model which is consistent with the notion that different PH pathophysiologies can emerge from different disease triggers, and results can vary in hosts with different genetic backgrounds.

To determine the clinical relevance of LTB_4 in human PAH, we examined the expression of LTA_4H in the lung tissue of PAH patients. In five out of six samples, LTA_4H expression was notably elevated in macrophages that were clustered around occluded vessels and in the

endothelial cells lining the lumen of the plexiform lesions. The fact that LTA_4H was seen in both macrophages and endothelial cells in advanced disease (as opposed to being principally localized in macrophages early in disease) upregulation of both direct and transcellular synthetic pathways; it is possible that in addition to LTB_4 being directly secreted by macrophages, LTA_4 was also secreted by these cells, and this LT was taken up in PAECs (which are poor LTA_4-producers) and converted in these increasingly abnormal occlusive intimal cells into LTB_4 [69]. Also, the serum concentration of LTB_4 was significantly elevated in PAH patients, especially in those with connective tissue disease-associated PAH (CTD-PAH). By contrast, six of eight patients with idiopathic PAH appeared to have normal LTB_4 levels. One explanation for these heterogeneous findings is that CTDs, such as systemic lupus erythematosus and systemic sclerosis, exhibit significant defects in Treg function and number (more so than, perhaps, idiopathic PAH patients) that may facilitate the inappropriate activation of macrophages including increased LTB_4 production [8, 70–72]. Thus, these new findings again lend support to the idea that different forms of PAH likely have unique pathogenic mechanisms [73, 74].

CysLTs in PAH

CysLTs are known to cause hypoxic pulmonary vasoconstriction [75], bronchoconstriction [76, 77], decreased lung compliance, and pulmonary edema [77]. The role of Cys-LTs has been well studied in pulmonary airways disease, but little is known about their role in PAH. Elevated levels of CysLTs were first reported in neonates with hypoxemia and pulmonary hypertension in 1983 [78]. The concentrations of eicosanoids, specifically TxB_2, 6-keto-PGF1α, PGD_2, PGE_2, LTB_4, LTC_4, and LTE_4, in bronchoalveolar lavage fluid (BALF) and blood of infants with persistent PH were also reported to be markedly increased. Furthermore, increased eicosanoids concentration correlated with poor clinical outcome of persistent pulmonary hypertension [79]. The continuing study of CysLTs in the airways may lend further insights into their role in the development of persistent PH as well as PAH.

Conclusions

This article has highlighted the growing information concerning how LTs may be participating in the development of certain forms of PAH. PAH remains an incurable and progressive disease. The last few decades have yielded a significantly deeper understanding of the pathobiology of PAH and have led to pharmacological advances, but there are challenges associated with these treatments, including the delivery systems, toxicity, frequent dosing schedules, variable efficacy, and cost [80]. Importantly, targeted immunotherapy as an adjuvant approach to current standard-of-care vasodilators has yet to be implemented. Anti-LT treatments may prove an effective complementary therapy for a subset of these patients.

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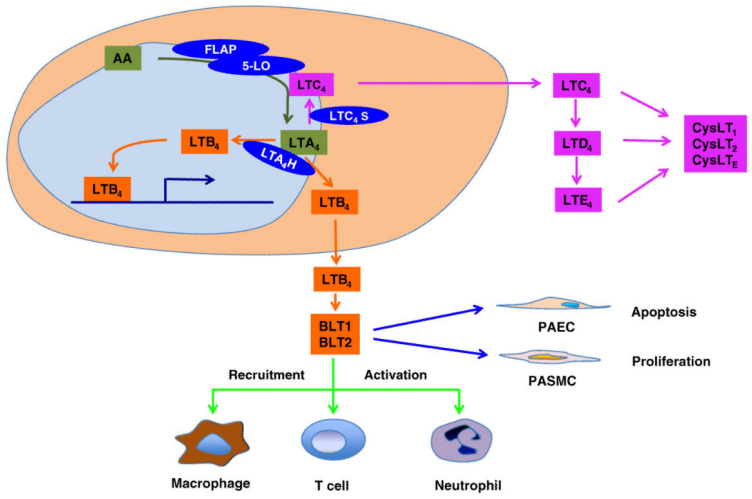

Fig. 1.

LT pathways and functions in PAH. LTs are synthesized from the arachidonic acid (AA) pathway, where 5-LO works together with FLAP on the perinuclear membrane that converts AA to LTA_4. LTA_4 is quickly metabolized to LTB_4 by LTA_4H, or is converted to LTC_4 by LTC_4S. LTC_4 undergoes sequential peptide cleavage of the glutathione moiety to form LTD_4 or LTE_4. LTB_4 may function as a transcriptional regulator in the nucleus or is transported out from the source cell and binding to its cognate receptors (BLT1 and BLT2) to initiate the downstream signaling. In PAH, elevated LTB_4 signaling around the disease arteriole results in the recruitment of the leukocytes. Recent data demonstrate that LTB_4 may also cause vascular remodeling by inducing the PAEC apoptosis and PASMC proliferation