

Review Article

Sphingosine kinase 1/sphingosine 1-phosphate signalling pathway as a potential therapeutic target of pulmonary hypertension

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Abstract: Pulmonary hypertension is characterized by extensive vascular remodelling, leading to increased pulmonary vascular resistance and eventual death due to right heart failure. The pathogenesis of pulmonary hypertension involves vascular endothelial dysfunction and disordered vascular smooth muscle cell (VSMC) proliferation and migration, but the exact processes remain unknown. Sphingosine 1-phosphate (S1P) is a bioactive lysophospholipid involved in a wide spectrum of biological processes. S1P has been shown to regulate VSMC proliferation and migration and vascular tension via a family of five S1P G-protein-coupled receptors (S1P₁-S1P₅). S1P has been shown to have both a vasoconstrictive and vasodilating effect. The S1P receptors S1P₁ and S1P₃ promote, while S1P₂ inhibits VSMC proliferation and migration *in vitro* in response to S1P. Moreover, it has been reported recently that sphingosine kinase 1 and S1P₂ inhibitors might be useful therapeutic agents in the treatment of empirical pulmonary hypertension. The sphingosine kinase 1/S1P signalling pathways may play a role in the pathogenesis of pulmonary hypertension. Modulation of this pathway may offer novel therapeutic strategies.

Keywords: Pulmonary hypertension, sphingosine 1-phosphate, endothelial dysfunction, vascular smooth muscle cell, pulmonary vascular remodelling

Introduction

Pulmonary hypertension is characterized by abnormal remodelling of small pulmonary arteries, which leads to increased pulmonary vascular resistance and right heart failure [1, 2]. Endothelial dysfunction is believed to be one of the early steps in the pulmonary hypertensive process, involving a decrease in the production of vasorelaxants, such as nitric oxide and prostacyclin, and an increase in the production of vasoconstrictors, such as endothelin [1-3]. This dysfunction leads to an increase in vascular smooth muscle cell (VSMC) proliferation, extracellular matrix deposition and hypercontractility.

Sphingosine 1-phosphate (S1P) is an important modulator of cell signalling that exerts its effects by binding to specific cell surface G-protein-coupled receptors. S1P is involved in

a wide variety of physiological processes including angiogenesis, cell proliferation and migration, inflammatory cell trafficking, cytokine production, cytoskeletal reorganization, endothelial barrier regulation and the control of vasomotor tone [4-8]. These effects of S1P can regulate the vascular tone and the proliferation and migration of VSMCs in the systemic circulation [9-12].

It is important to establish the role of the S1P signalling pathway in the pathogenesis of pulmonary hypertension to understand how modulation of this pathway may offer novel therapeutic strategies. The exact processes involved in the pathogenesis of the disease remain unknown. The aim of this review article is to present an overview of the current understanding of the S1P signalling pathway in the pathogenesis of pulmonary hypertension.

S1P biosynthesis and signalling

Sphingolipids are ubiquitous components of the lipid bilayer of eukaryotic cells. As in the case of glycolipids, sphingolipid metabolism is regulated by numerous agonists that generate signalling molecules, including ceramide (N-acyl sphingosine), sphingosine, and S1P [13]. Ceramide, the backbone of sphingolipids, is produced by *de novo* synthesis and turnover of sphingolipids. After removal of the sphingolipid head groups during catabolism, deacylation of ceramide by ceramidases yields sphingosine [14]. Sphingosine is phosphorylated by type 1 and type 2 sphingosine kinases (SphK1 and SphK2) to form S1P. S1P can undergo degradation by one of two pathways: it may be converted to sphingosine by reversible dephosphorylation mediated by a variety of phosphohydrolases; or it may form ethanolamine phosphate and hexadecanol after undergoing irreversible cleavage mediated by S1P lyase [13].

Sphingosine 1-phosphate is a bioactive lysophospholipid that mediates many important cellular processes, including proliferation, migration, differentiation, cytoskeletal rearrangements, motility, angiogenesis, calcium mobilization, lymphocyte trafficking, and immune function [5-8]. Most cells have the enzymatic machinery to synthesize S1P. In serum and plasma, the S1P concentrations range about between 200 and 900 nM, but these values are likely to change under different pathological conditions. Sources of S1P in plasma include red blood cells [8], platelets [15], and endothelial cells [16]. S1P levels are reported to be 8-fold greater in the lungs than elsewhere [17].

Many actions of S1P are mediated via five S1P G-protein-coupled receptor subtypes (S1P₁-S1P₅) [13, 18, 19]. Although S1P receptors are expressed in almost every cell type, S1P₁, S1P₂ and S1P₃ are predominant in the vascular system [20]. Reverse transcription-polymerase chain reaction analysis showed that S1P₁ and S1P₃ messenger RNA (mRNA) were present in both pulmonary artery endothelial cells and pulmonary artery VSMCs, while S1P₂ mRNA was confined to pulmonary artery VSMCs [21].

S1P in endothelial dysfunction

Pulmonary vasoconstriction is believed to be an early step in the pulmonary hypertensive

process. Excessive vasoconstriction is related to endothelial dysfunction [3], and endothelial dysfunction is characterized by decreased levels of nitric oxide (NO) [22] and prostacyclin [23], which occur concomitantly with increased endothelin-1 levels [24]. NO is a potent pulmonary arterial vasodilator and a direct inhibitor of platelet activation and VSMC proliferation. The reduced NO bioavailability in pulmonary hypertension may be due to decreased endothelial NO synthase (eNOS) expression, inhibition of eNOS enzymatic activity or inactivation of NO by superoxide anion. Prostacyclin acts synergistically with NO to induce VSMC relaxation, inhibit platelet activation and prevent VSMC migration and proliferation.

S1P has been shown to inhibit inducible NOS expression and interleukin-1 β -induced NO production in rat VSMCs [25]. In contrast, others have found that NO and prostaglandin I₂ synthesis were stimulated by S1P in vascular endothelial cells and VSMCs *in vitro* [26-29]. A study by Morales-Ruiz *et al.* found that S1P activates eNOS downstream of S1P₁ in bovine microvascular endothelial cells *in vitro*, suggesting that S1P drives pulmonary vasorelaxation [26].

It is important to note that S1P has been found to induce vasoconstriction instead of vasodilation in some experimental systems. Studies have suggested that S1P exerts a vasoconstrictive response in the pulmonary vasculature through increasing the tension in isolated conduit pulmonary arterial segments [30, 31]. S1P induced dose-dependent pulmonary vasoconstriction in the mouse via S1P₂ and a Rho-kinase-mediated signal transduction pathway [32]. In addition, a study assessing vasoconstriction in normoxic and hypoxic isolated rat lungs suggested a significant role for S1P₄ in S1P-induced vasoconstriction [33]. S1P may therefore play a role in the increased pulmonary vascular resistance associated with pulmonary hypertension. Further research is needed to determine the detailed mechanisms underlying the S1P-mediated regulation of vascular tone.

SphK1/S1P pathway in pulmonary vascular remodelling

Vascular remodelling is characterized by medial hypertrophy due to enhanced VSMC proliferation and migration, attenuated apoptosis and over proliferation of endothelial cells, all of

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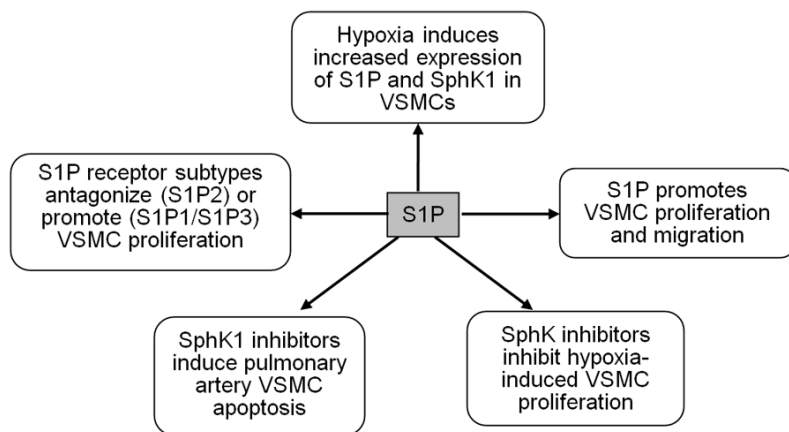


Figure 1. Roles of the sphingosine-1-phosphate (S1P) signalling pathway in pulmonary artery vascular smooth muscle cells (VSMCs). SphK1; sphingosine kinase type 1.

which can result in lumen obstruction and pulmonary hypertension. The proliferation and migration of VSMCs induces an increase in the deposition of VSMCs on the vascular matrix [34]. An increase in extracellular matrix components and myofibroblast formation are also involved in the remodelling process [35, 36].

The significantly increased expression of S1P in VSMCs subjected to hypoxia contributes to the proliferation and migration of these cells [37]. In addition, the growth of hypoxia-induced VSMCs is significantly inhibited by the competitive SphK inhibitor, D-erythro-N,N-dimethylsphingosine (DMS), indicating that intracellular S1P may play a regulatory role in key signalling in response to hypoxia [38]. DMS inhibits VSMC growth by inhibition of both extracellular signal-regulated kinase-1/2 (ERK-1/2) and phosphorylated protein kinase B (Akt) signalling, which are involved in regulating cell growth and survival [39]. Chronic hypoxia increases the phosphorylation of ERK-1/2 in response to S1P [40]. The enhancing effect of chronic hypoxia on the responsiveness of ERK1/2 to S1P might be related to the regulation of hypoxia-inducible factors, which are activated by ERK1/2 and control the expression of growth factors such as vascular endothelial growth factor. These growth factors can in turn promote vascular remodelling. Hypoxia was also shown to induce increased levels of SphK1 in human pulmonary artery VSMCs, but has no effect on S1P₁ or S1P₃ mRNA levels [40]. Further research is required to elucidate S1P-induced VSMC proliferation in response to hypoxia.

Activation of S1P₂ antagonizes S1P-induced VSMC proliferation and migration via a Rho-dependent pathway, whereas S1P₁/S1P₃ activation promotes VSMC proliferation and *in vitro* phenotypic modulation (**Figure 1**) [11, 41-43]. S1P₁, S1P₂ and S1P₃ are coupled to different and opposing signalling cascades. S1P₁ couples exclusively with members of the G_α family, and S1P₂ and S1P₃ couple to multiple G proteins including G_qα and G_{12/13}α [44]. S1P stimulates activation of phosphatidylinositol

3-kinase/Akt and ERK via S1P₁, and RhoA via S1P₂ [45, 46]. S1P also induces the release of calcium from intracellular stores via S1P₃ [45, 46].

Basic fibroblast growth factor is involved in the physiological activities of VSMCs, including protection from apoptosis, promotion of proliferation and migration. In addition, basic fibroblast growth factor upregulates S1P₁ in human pulmonary artery VSMCs [47], which may contribute to pulmonary vascular remodelling.

Studies have examined the effects of the S1P signalling pathway on pulmonary artery cells, and found that S1P increased Rho kinase activity in a time-dependent manner in pulmonary artery VSMCs [32]. Rho kinase has been shown to play an important role in the pathogenesis of pulmonary hypertension [21, 48, 49]. Research has also highlighted the role of SphK1 in the immunological pathogenesis of pulmonary arterial hypertension. Reduction of SphK1 activity increased pulmonary vascular hyperresponsiveness and contributed to the development of inflammation-associated pulmonary hypertension [50], and inhibition of SphK1 induced apoptosis in pulmonary artery VSMCs [51].

Empirical studies in which SphK1 and S1P2 inhibitors attenuate PH

It has recently been suggested that SphK1 and S1P2 inhibitors might be useful therapeutic agents in the treatment of pulmonary hypertension [52]. SphK1 and S1P were significantly

increased in the lungs of experimental hypoxia-induced pulmonary hypertension mice and from patients with pulmonary hypertension. SphK1 deficient (SphK1^{-/-}) mice were protected from hypoxia-induced pulmonary hypertension [52]. SKI2, the inhibitor of both SphK1 and SphK2 prevented the development of hypoxia-induced pulmonary hypertension and inhibited pulmonary vascular remodeling [52]. Moreover, JTE013, the S1P2 inhibitor prevented and reversed the development of hypoxia-induced pulmonary hypertension and pulmonary vascular remodeling [52]. Thus, these data suggested that Sphk1/S1P signalling pathway played a critical role in the development of hypoxia-induced pulmonary hypertension.

Conclusions

This review summarizes the roles of the S1P signalling pathway in the pathogenesis of pulmonary hypertension and suggests a new approach for the treatment of the disease. Evidence from *in vivo* and clinical studies is limited, and further studies are necessary to fully examine the potential therapeutic implications of targeting S1P and its receptors in pulmonary hypertension. Modulating the S1P signalling pathway may provide novel therapeutic strategies for pharmacological intervention in pulmonary hypertension.

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Disclosure of conflict of interest

None.

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