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REVIEW

The Antifibrotic Effects of Inhaled Treprostinil: An Emerging Option for ILD

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ABSTRACT

Interstitial lung diseases (ILD) encompasses a heterogeneous group of parenchymal lung diseases characterized by variable amounts of inflammation and fibrosis. The targeting of fibroblasts and myofibroblasts with antifibrotic treatments is a potential therapeutic target for these potentially fatal diseases. Treprostinil is unique among the prostacyclin mimetics in that it has distinct actions at additional prostaglandin receptors. Preclinical and clinical evidence suggests that treprostinil has antifibrotic effects through the activation of the prostaglandin E receptor 2 (EP_2), the prostaglandin D receptor 1 (DP_1), and peroxisome proliferator-activated receptors (PPAR). In vivo studies of EP_2 and the DP_1 have found that administration of treprostinil resulted in a reduction in cell proliferation, reduced collagen secretion and synthesis, and reduced lung

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inflammation and fibrosis. In vitro and in vivo studies of PPARβ and PPARγ demonstrated that treprostinil inhibited fibroblast proliferation in a dose-dependent manner. Clinical data from a post hoc analysis of the INCREASE trial found that inhaled treprostinil improved forced vital capacity in the overall population as well as in idiopathic interstitial pneumonia and idiopathic pulmonary fibrosis subgroups. These preclinical and clinical findings suggest a dual benefit of treprostinil through the amelioration of both lung fibrosis and pulmonary hypertension.

Keywords: Antifibrotic; Idiopathic pulmonary fibrosis; Interstitial lung diseases; Pulmonary fibrosis

Key Summary Points
Treprostinil is approved for the treatment of pulmonary arterial hypertension and pulmonary hypertension associated with interstitial lung disease.
The antifibrotic effects of treprostinil are mediated through the activation of the prostaglandin E receptor 2, the prostaglandin D receptor 1, and peroxisome proliferator-activated receptors.
Preclinical and clinical data provide evidence for the antifibrotic effects of treprostinil.
Treprostinil may have a role in mitigating the effects of fibrosis caused by vascular remodeling, cytokine overexpression, and alveolar wall thickening.

DIGITAL FEATURES

This article is published with digital features, including an infographic, to facilitate understanding of the article. To view digital features for this article go to https://doi.org/10.6084/m9.figshare.20073086.

INTRODUCTION

The broad category of interstitial lung diseases (ILD) encompasses a heterogeneous group of parenchymal lung diseases that are characterized by varying amounts of inflammation and fibrosis of the bronchioles and interstitium of the lungs [1, 2]. Lung fibrosis may be the result of inflammation in certain ILDs, while in others it may result from fibroblastic proliferation. Fibrosis tends to be progressive and invariably results in the destruction of the normal extracellular lung matrix with architectural distortion [3–5]. The resulting fibrotic tissue hinders gas exchange and reduces lung compliance, leading to progressive shortness of breath, functional impairment, and ultimately respiratory failure and death [6–8].

Numerous therapies have been used off-label to treat ILDs, but there are only four therapies specifically approved for distinct ILD indications. Pirfenidone and nintedanib, which have antifibrotic properties, are both approved for the treatment of idiopathic pulmonary fibrosis (IPF), while nintedanib carries a broader label to include ILD due to scleroderma (SSc-ILD) and patients with a progressive fibrotic phenotype [9, 10]. More recently, the interleukin-6 (IL-6) receptor antagonist tocilizumab was approved for SSc-ILD [11]. There have been numerous clinical trials of pulmonary vasodilators for the treatment of ILD, which were based on the hypothesis that these drugs may ameliorate the vascular component of ILD [12]. Unfortunately, studies of endothelin receptor antagonists and phosphodiesterase 5 inhibitors have been largely negative, with a few even suggesting harm in patients with IPF.

One exception is sildenafil which has demonstrated a quality-of-life benefit and a trend toward improvement in mortality in the STEP-IPF trial [13] as well as lower risk of forced vital capacity (FVC) decline when combined with nintedanib in the INSTAGE trial [14]. These results suggested a potential role for pulmonary vasodilators for ILDs and set the stage

for the landmark INCREASE trial, which was a randomized controlled study evaluating inhaled treprostinil in pulmonary hypertension associated with ILD (PH-ILD) [15]. This study met its primary endpoint, demonstrating a 31-m improvement in 6-min walk distance (6MWD), leading to US Food and Drug Administration approval of inhaled treprostinil as the first and only treatment for PH-ILD on April 1, 2021. Interestingly, a post hoc analysis of the INCREASE trial suggested that inhaled treprostinil was associated with an improvement in FVC, which has never been demonstrated in any well-controlled study of ILD [16]. There may be several reasons for this FVC improvement, but one potential mechanism is the antifibrotic property of treprostinil; treprostinil's role in reducing fibrosis and thereby acting as a potential treatment option for pulmonary fibrosis has been illuminated through recent studies and will be discussed herein. A summary of the preclinical and clinical studies attesting to the antifibrotic effects of treprostinil is provided in Table 1. This review is based on previously conducted studies and does not contain any new studies with human participants or animals performed by any of the authors.

Pathophysiology

Pathologic vascular findings are common in ILD and may consist of changes to arteries, arterioles, and venules, as well as the destruction of capillary beds [17]. Increased vascular resistance in pre-capillary vessels leads to increased pulmonary arterial pressure and pulmonary hypertension [18].

Cytokines and cytokine-like molecules, such as transforming growth factor beta 1 (TGFβ1), platelet-derived growth factor (PDGF), and endothelin-1 (a potent vasoconstrictor) [19], have been shown to regulate pulmonary fibrosis [20, 21]. Their overexpression may contribute to vascular remodeling and increased extracellular matrix deposition, leading to increased fibrosis and increased pulmonary arterial pressures [7, 20]. Impaired angiogenesis in fibrotic lung tissue may impact vascular remodeling further and contribute to the rise in pulmonary arterial pressures [22].

Given the complex interplay between the lung interstitium and pulmonary vasculature in ILD, the intersection between the two is an area of emerging interest.

TREPROSTINIL MECHANISM OF ACTION

Treprostinil is currently approved for the treatment of pulmonary arterial hypertension (PAH; WHO Group 1) and PH-ILD (WHO Group 3) to improve exercise ability [23]. It is a full prostacyclin receptor (IP) agonist and also has a high affinity for prostaglandin E receptor 2 (EP_2) and the prostaglandin D receptor 1 (DP_1) [24]. Figure 1 depicts the mechanism of action of treprostinil and physiologic effects resulting from each receptor.

Upon activation, IP couples with adenylate cyclase to convert adenosine triphosphate to cyclic adenosine monophosphate (cAMP), activating protein kinase A [25]. A secondary pathway involves activation of peroxisome proliferator-activated receptor β (PPARβ), activating anti-inflammatory mechanisms including activation of the retinoid X receptor to drive gene transcription, suppression of B cell lymphoma 6, and suppression of protein kinase C-α [25]. Both pathways lead to vasorelaxation, reduced thrombosis from platelet inhibition, reduced vascular remodeling and inflammation, and contribute to treprostinil's antifibrotic effects [25].

When activated, IP, EP_2, and DP_1 induce a G protein-coupled cascade that results in an increase in protein kinase A which produces treprostinil's therapeutic effects of vasodilation [26, 27], platelet inhibition [28–30], antiproliferation [31–35], anti-inflammation, and antifibrosis [23, 24, 36]. Activation of these receptors has been shown to inhibit fibroblast proliferation, collagen secretion, and fibroblast-to-myofibroblast differentiation. The following section will review the specific receptors, transcription factors, growth factors, and physiology possibly involved in the antifibrotic process of treprostinil.

Table 1 Preclinical and clinical evidence for the antifibrotic effects of treprostinil

Study	Methods and purpose	Main findings
Preclinical		
In vitro		
Wilborn 1995 [42]	Comparison of lung fibroblasts isolated from patients with IPF and patients undergoing resectional surgery for lung cancer	Demonstrated diminished capacity to synthesize PGE_2 and express COX-2
Kolodsick 2003 [33]	Normal human fetal lung fibroblasts were examined to determine if PGE_2 could modulate the transition of lung fibroblasts to myofibroblasts	PGE_2 inhibits the transition of fibroblasts to myofibroblasts
Burgess 2004 [37]	Cultured human airway smooth muscle cells from asthmatic and nonasthmatic patients tested to see if PGE_2 can inhibit proliferation and identify the receptors involved	EP_2 receptor is responsible for the antiproliferative effects of PGE_2 in airway smooth muscle cells
Moore 2005 [34]	Fibroblasts from bleomycin-treated C57BL/6 mice were analyzed for prostanoid receptor changes	Loss of PGE_2 suppression is associated with reduced expression of EP_2 receptors. This resulted in fibroblasts that were unresponsive to PGE_2
White 2005 [32]	Cultured normal human fetal lung fibroblasts and embryonic *pten*-null murine fibroblast cells were analyzed to analyze the effects of treatment with PGE_2	Treatment with PGE_2 inhibited fibroblast migration via the EP_2 receptor, leading to increased PTEN and diminished fibroblast migration
Ali 2006 [44]	Cultured lung tissue from IP- or PPARβ-deficient murine models was examined to assess the role of IP and PPARβ as therapeutic agents for PH	Antiproliferative effects of treprostinil are mediated by PPARβ, not IP
Ali 2006 [29]	Blood samples from healthy patients were tested to investigate the presence and function of PPARβ in human platelets	Activation of PPARβ was found to have anti-inflammatory effects and PPARγ was found to inhibit cell proliferation
Falcetti 2007 [45]	HEK-293 cells were transfected with human IP to investigate whether PGI_2 analogues regulate PPARγ	Prostacyclin analogues activated PPARγ in an IP-dependent manner
van den Brule 2010 [43]	Cultured lung fibroblasts from bleomycin-treated female C57BL/6 mice to examine the effects of a DP agonist	Activation of DP_1 receptors reduced lung inflammation and fibrosis
Ayabe 2013 [55]	Primary human fetal lung fibroblasts were stimulated with TGFβ and treated with PGD_2, DP receptor agonists, DP receptor antagonists, or CRTH2 to assess the effects on collagen synthesis and secretion	PGD_2 inhibits TGFβ induced collagen secretion via activation of the DP receptor and intracellular cAMP accumulation

Table 1 continued

Study	Methods and purpose	Main findings
Dagouassat 2013 [40]	Primary lung fibroblasts from patients with COPD, male C57BL/6 mice, and $p53^{-/-}$ mice to analyze the role of PGE_2 in inducing senescence and inflammation	COPD lung fibroblasts had higher levels of EP_2 and EP_4 receptors than healthy smoking and non-smoking controls and displayed increased senescent markers. $p53^{-/-}$ murine models showed that PGE_2 is responsible for this increased senescence and inflammation
Safholm 2015 [56]	Cultured healthy human lung tissue samples were treated PGE_2 with or without an EP_4 receptor antagonist to characterize the effects of PGE_2	EP_2 receptors were shown to inhibit mast cell-mediated bronchoconstriction
Horikiri 2017 [41]	PGE-MUM levels were analyzed via radioimmunoassay in controls and patients with lung diseases. Human bronchial epithelial cells and lung fibroblast samples were treated with TGFβ to analyze its role in EP_2 receptor expression	PGE-MUM levels were increased in patients with chronic lung fibrosis and were correlated with fibrosis scores
Lambers 2018 [36]	Human peripheral lung fibroblasts were stimulated with PDGF or TGFβ1, or both and incubated with treprostinil, forskolin, DDA, or vehicle to investigate their effects on PDGF-BB and TGFβ activated intracellular signaling	Treprostinil activated cAMP, preventing PDGF-BB-induced proliferation and TGFβ1 secretion
Patel 2018 [38]	Human PASMCs from patients with PAH were treated with agonists, antagonists, or EP_2 receptor siRNAs to assess the effects on receptor expression, cell proliferation, and cAMP	EP_2 receptors were elevated in PAH cells and treprostinil demonstrated EP_2-dependent antiproliferative actions
Roberts 2018 [46]	Normal human lung fibroblasts were used to test the function of select Gs-coupled GPCR agonists and their ability to inhibit fibroblast proliferation and differentiation	Formoterol, PGE_2, treprostinil, and forskolin all elicited maximal cAMP responses. BAY60-6583 and MRE-269 fully inhibited fibroblast proliferation and differentiation and were partial cAMP agonists. The magnitude of cAMP response was not predictive of antifibrotic efficacy
Blumer 2021 [47]	Fibroblasts from human lung tissue from patients with end-stage ILD were isolated and treated with TGFβ1 or TGFβ1 + treprostinil	Phosphorylation of ERK1/2 MAPK was significantly reduced with treprostinil. Treatment with treprostinil also increased the expression of DUSP1, which was decreased by TGFβ1. This resulted in a concentration-dependent reduction in TGFβ1-induced proliferation

Table 1 continued

Study	Methods and purpose	Main findings
In vivo		
Corboz 2018 [49]	Rats with bleomycin-induced pulmonary fibrosis were intranasally administered INS1009 to evaluate potential antifibrotic effects and cultured human lung fibroblasts were treated with treprostinil to determine the effects on genes associated with collagen synthesis and secretion	INS1009 dose-dependently reduced lung hydroxyproline, demonstrating an antifibrotic effect of inhaled treprostinil by mechanisms likely involving suppression of collagen production from lung fibroblasts
Nikitopoulou 2018 [50]	Mice with bleomycin-induced injury were treated with orotracheally administered treprostinil or vehicle to determine if treprostinil has downstream effects on inflammation and pulmonary fibrosis	Treprostinil reduced bleomycin-induced lung dysfunction and attenuated lung injury compared with mice receiving placebo. Mice treated with inhaled treprostinil showed less inflammation, focal alveolar thickening, and reduced collagen deposition
Clinical		
Nathan 2021 *Lancet* [16]	Evaluated the change in FVC in the overall population from the INCREASE study and subgroup analysis	Treatment with inhaled treprostinil resulted in FVC improvements in patients with PH-ILD. Patients with IIP and IPF also demonstrated FVC improvements
Nathan 2021 *Chest* [52]	Comparison of lung function changes in the INCREASE and TRIUMPH studies	Findings suggest that the pulmonary function test response to inhaled treprostinil differs mechanistically between PAH and PH-ILD, with significant improvements seen in % predicted FVC in patients with PH-ILD but not patients with PAH
Waxman 2021 [15]	Evaluated the efficacy and safety of inhaled treprostinil in patients with PH-ILD	Treatment with inhaled treprostinil significantly improved exercise capacity for patients with PH-ILD and was associated with a lower risk of clinical worsening
TETON (NCT04708782) [57]	Evaluate the safety and efficacy of inhaled treprostinil in patients with IPF	Ongoing

ATP adenosine triphosphate, *cAMP* cyclic adenosine monophosphate, *COPD* chronic obstructive pulmonary disorder, *COX-2* cyclooxygenase-2, *CRTH2* chemoattractant receptor–homologous molecule expressed on Th2 cells, *DDA* dideoxyadenosine, *DP* prostaglandin D receptor, *EP_2* prostaglandin E receptor 2, *FVC* forced vital capacity, *GPCR* G protein-coupled receptors, *IP* prostacyclin receptor, *IIP* idiopathic interstitial pneumonia, *IPF* idiopathic pulmonary fibrosis, *IIP* idiopathic interstitial pneumonia, *mPGES-1* microsomal prostaglandin E_2 synthase 1, *MAPK* mitogen-activated protein kinase, *mRNA* messenger ribonucleic acid, *PAH* pulmonary arterial hypertension, *PASMC* pulmonary arterial smooth muscle cell, *PGE_2* prostaglandin E_2, *PGE-MUM* prostaglandin E major urinary metabolite, *PH* pulmonary hypertension, *PH-ILD* interstitial lung disease with pulmonary hypertension, *PPARβ* peroxisome proliferator-activated receptor β, *PTEN* phosphatase and tensin homolog on chromosome ten, *siRNA* small interfering ribonucleic acids, *SP* substance P

PRECLINICAL DATA TO SUPPORT THE ANTIFIBROTIC EFFECTS OF TREPROSTINIL

Role of the EP_2 Receptor

The EP_2 receptor, an important receptor for its role in fibrosis, is responsible for the antiproliferative effects of prostaglandin E_2 (PGE_2) in airway smooth muscle cells [37], In PAH, EP_2 receptor expression is upregulated compared to lung tissue samples from healthy controls, while IP expression is decreased [38]. This observation may imply that EP_2 is upregulated as a consequence of disease, or that pulmonary disease may negatively impact IP expression [38–41]. Therefore, it is important to consider the role of EP_2 as a negative modulator of vascular tone, proliferation, and fibrosis.

Experiments have shown that treprostinil's antifibrotic effects are independent from IP receptors, being more dependent on other receptors such as EP_2 and DP_1. When human pulmonary arterial smooth muscle cells (PASMCs) were incubated with an IP receptor antagonist (R01138452), the antiproliferative effects of a non-prostanoid IP receptor agonist (MRE-269) were abolished [38]. However, increasing concentrations of treprostinil continued to produce a marked reduction in cell proliferation despite the presence of the IP receptor antagonist [38]. Incubating PASMCs with an EP_2 receptor antagonist resulted in the opposite effect, significantly reducing the antiproliferative effects of treprostinil, supporting the activation of EP_2 by treprostinil [38].

Activation of EP_2 receptors has a range of inhibitory effects on fibroblast function that could have beneficial effects in patients with pulmonary fibrosis [39]. Through different in vitro experiments, fibroblast treatment with PGE_2 has displayed inhibition of fibroblast-to-myofibroblast transition and suppression of fibroblast proliferation and reduced collagen synthesis [33, 34]. Because patients with IPF have a diminished capacity to synthesize PGE_2 [42], supplementation with PGE_2 or a small molecule that activates EP_2 receptors, such as treprostinil, could therefore have therapeutic potential for patients.

Role of the DP_1 Receptor

In addition to EP_2, treprostinil also has a high affinity for the lesser-known DP_1, which also contributes to the antifibrotic action of treprostinil through mechanisms similar to EP_2.

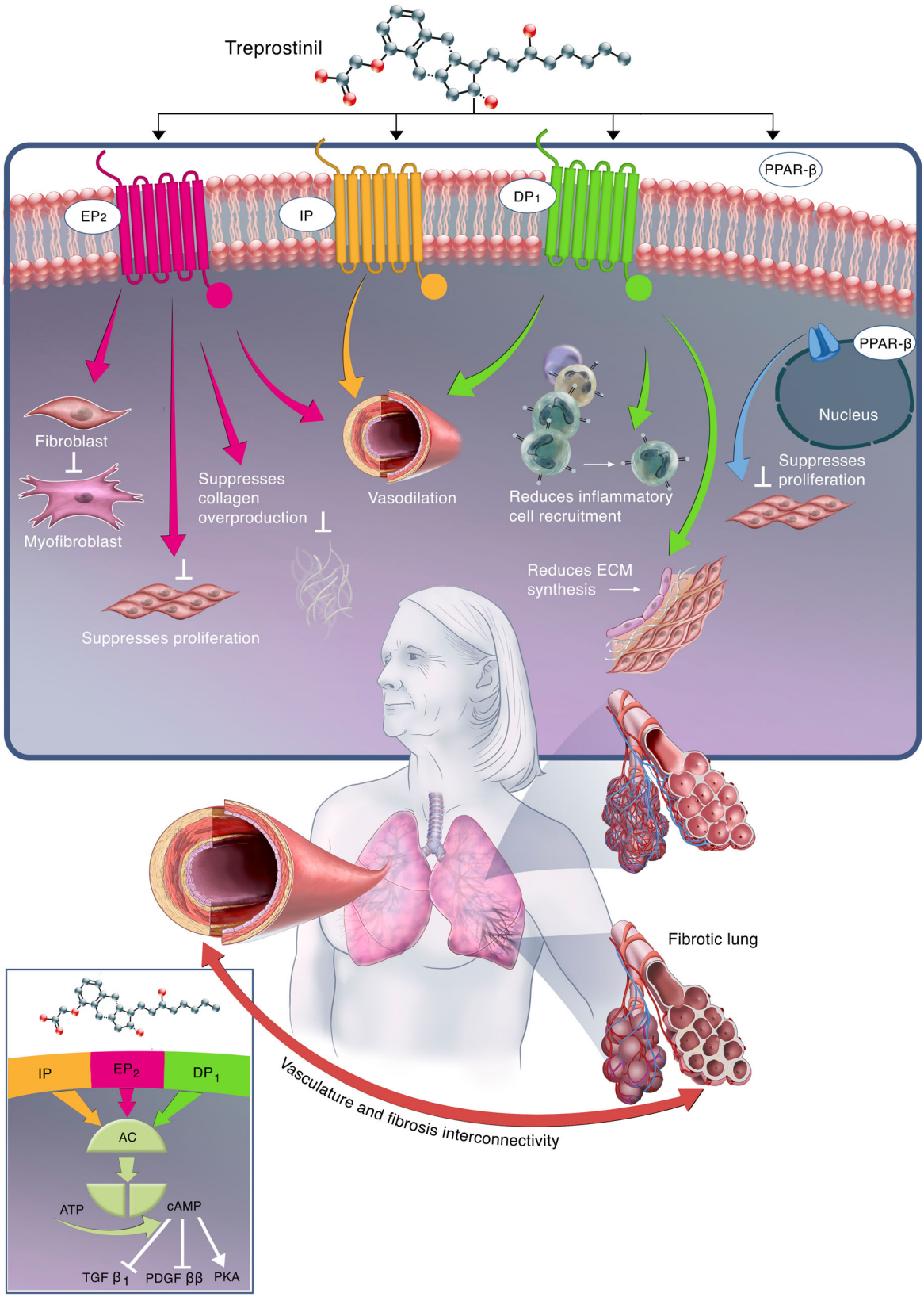
Further experiments showed that activation of DP_1 receptors reduced lung inflammation and fibrosis. C57BL/6 mice were treated with 500 nmol/kg of BW245C, a DP_1 agonist, or placebo for 2 days before receiving bleomycin treatment, followed by continued treatment with BW245C or placebo three times a week [43]. Lactate dehydrogenase, a measurement of cell damage, was significantly decreased with BW245C compared with placebo. Treatment with BW245C also reversed bleomycin-induced lymphocyte recruitment and significantly reduced collagen accumulation [43], Overall, these results show that activation of DP_1 significantly decreases both inflammatory cell recruitment and pulmonary collagen accumulation, thus providing evidence for the involvement of the DP_1 receptor in reducing fibrosis.

As treprostinil has a high affinity for DP_1, it follows that treatment with treprostinil would result in decreasing inflammatory cell recruitment and collagen accumulation through the activation of DP_1 receptors.

Role of PPARs

PPARs are a family of transcription factors that influence lipid and energy metabolism, epidermal wound repair, inflammation responses, and atherosclerotic plaque formation. There are three major isoforms, PPARα, PPARβ, and PPARγ. The role of PPARs in the antifibrotic effect of treprostinil has been described though is still being explored. A study by Ali et al. showed that treprostinil increased PPARβ which resulted in inhibition of fibroblast proliferation [44].

The effect of treprostinil on PPARγ is not as well defined. A 2006 study by Ali et al. found


Treprostinil
EP2
IP
DP1
PPAR-β
Fibroblast
Myofibroblast
Suppresses collagen overproduction
Vasodilation
Reduces inflammatory cell recruitment
Nucleus
PPAR-β
Suppresses proliferation
Reduces ECM synthesis
Suppresses proliferation
Fibrotic lung
Vasculature and fibrosis interconnectivity
IP
EP2
DP1
AC
ATP
cAMP
TGF β1
PDGF ββ
PKA

◀**Fig. 1** Schematic depicting the impact of treprostinil on the relationship between vascular remodeling, cytokine overexpression, and the development of fibrosis within the lungs. Treprostinil binds and activates the EP2, IP, and DP1 receptors and activates the PPARβ receptors to produce antifibrotic effects. Activation of the EP2, IP, and DP1 receptors leads to vasodilation. Activation of EP2 additionally inhibits fibroblast to myofibroblast differentiation, suppresses fibroblast proliferation, and suppresses collagen overproduction. Activation of DP1 additionally reduces inflammatory cell recruitment and reduces extracellular matrix synthesis. Activation of the nuclear receptor PPARβ leads to suppressed fibroblast proliferation. Collectively, treprostinil activates EP2, IP, DP1, and PPARβ and causes vasodilation, reduced vascular remodeling, reduced fibroblast activity, proliferation and collagen deposition, and reduced inflammation, thereby promoting antifibrotic activity. Mechanistically, when IP, EP2, and DP1 are activated, G protein-coupled signaling triggers adenylyl cyclase and converts ATP to cAMP, which drives the activation of TGFβ1, PDGFββ, and PKA, leading to therapeutic effects. Treprostinil activation of PPARβ drives therapeutic effects via an anti-inflammatory pathway, leading to activation of retinoid X receptor, suppression of B cell lymphoma 6, and suppression of protein kinase C-α (not shown). *IP* prostacyclin receptor, *EP2* prostaglandin E type 2 receptor, *DP1* prostaglandin D type 1 receptor, *PPARβ* peroxisome proliferator-activated receptor β, *ECM* extracellular matrix, *AC* adenylyl cyclase, *ATP* adenosine triphosphate, *cAMP* cyclic adenosine monophosphate, *TGFβ1* transforming growth factor β1, *PDGFββ* platelet-derived growth factor ββ, *PKA* protein kinase A

that PPARγ was not activated by treprostinil [29], but studies by Falcetti et al. found that prostacyclin analogues do activate PPARγ in an IP-dependent manner [45]. These findings were confirmed using the PPARγ antagonist GW9662, which significantly reduced the antiproliferative effects of treprostinil. The PPAR pathway is intriguing and presents an area warranting further exploration.

Cytokines and Growth Factors

When found in healthy tissue, TGFβ1 and PDGF have antiproliferative and apoptotic effects; however, in patients with PAH, TGFβ1 causes excess proliferation of PASMCs [24]. Additionally, patients with PAH have elevated levels of growth factors, including TGFβ1, PDGF, vascular endothelial growth factor, epithelial growth factor, and angiopoietin [24].

A study by Lambers et al. investigated the effects of treprostinil on PDGF and TGFβ1 intracellular signaling in fibroblasts cells from patients with IPF [36]. The study demonstrated that PDGF-BB and TGFβ1 induced α-smooth muscle actin, a marker of activated fibrogenic cells, and that this effect was dose-dependently reduced with treprostinil.

Furthermore, these inhibitory effects on PDGF and TGFβ1 appear to be mediated by the increased activation of cAMP; a downstream effect of relaxant prostanoid receptor activation [36]. Together, these results show that treprostinil can prevent PDGF and TGFβ1-mediated profibrotic proliferation and extracellular matrix synthesis in IPF fibroblasts.

Further research into the effects of prostacyclin agonists elucidated some of the mechanisms behind these downstream effects of cAMP. Roberts et al. found that PDGF-induced fibroblast proliferation was inhibited by treatment with the prostacyclin agonists treprostinil, MRE-269, and iloprost [46]. Treprostinil partially inhibited both processes while generating maximal cAMP; however, iloprost did not block PDGF-induced proliferation despite also causing maximal cAMP signaling.

Recent research has shown that the microRNAs negatively regulate the downstream fibrotic effects of TGFβ1, but clusters of microRNAs are dysregulated in patients with IPF and other fibrotic lung diseases, leading to increases in Erk1/2 mitogen-activated protein kinase [47]. When lung tissue samples from patients with end-stage ILD were treated with treprostinil, this dysregulation was overcome in a concentration-dependent manner through the upregulation of DUSP1, an Erk1/2 mitogen-activated protein kinase inhibitor, reducing TGFβ1-induced proliferation [47].

BLEOMYCIN LUNG MODELS

To further evaluate the antifibrotic effects of treprostinil, animal models were simulated in two significant studies using bleomycin-induced pulmonary fibrosis models [48]. In a study by Corboz et al., rats were treated with 10, 30, or 100 μg/kg of INS1009, an inhaled prodrug of treprostinil, inhaled phosphate buffer saline (PBS), or 100 mg/kg pirfenidone administered orally twice daily for 17 days, beginning 10 days post-bleomycin challenge [49].

All doses of INS1009 significantly reduced hydroxyproline, a marker of collagen deposition and synthesis, in the lungs in a dose-dependent manner. The lowest dose of treprostinil, 10 μg/kg, produced a 44% reduction in hydroxyproline levels, while the medium and high doses resulted in 68% and 88% reductions, respectively. Orally administered pirfenidone also reduced hydroxyproline, but to a lesser extent than the upper doses of treprostinil (60%) [49].

In the same study, bleomycin-induced pulmonary fibrosis was also analyzed by measuring changes in lung mass to assess fibrotic changes and collagen deposition. Bleomycin-challenged animals treated with inhaled PBS had a significant increase in right caudal lung mass; whereas, rats treated with an inhaled prodrug of treprostinil exhibited a dose-dependent reduction in right caudal lung mass. These results demonstrate an antifibrotic effect of inhaled treprostinil in a rat model of bleomycin-induced pulmonary fibrosis by mechanisms involving the suppression of collagen production from lung fibroblasts [49].

Additional animal model studies by Nikitopoulou et al. evaluated the effect of inhaled treprostinil on inflammation, pulmonary fibrosis, and vascular remodeling in a bleomycin-induced model of pulmonary fibrosis [50]. Inhaled treprostinil or placebo were administered to mice twice daily beginning 1 day prior to bleomycin or saline challenge. Impaired breathing and lung function were assessed by measuring tissue elasticity and static compliance on days 7, 14, and 21. Daily treatment with treprostinil reduced bleomycin-induced lung dysfunction compared with mice receiving placebo. A statistically significant change in elasticity was achieved at all three time points, and on day 21 for static compliance. Histological analyses of the mice showed that inhaled treprostinil attenuated lung injury. Tissue samples displayed less inflammation as well as a reduction of alveolar wall thickening and collagen deposition [50].

Overall, inhaled treprostinil (40 μg/kg twice daily) maintained lung function and prevented bleomycin-induced lung injury, fibrosis, and vascular remodeling. The authors concluded that these findings suggest treprostinil potentially has therapeutic efficacy in pulmonary fibrosis and pulmonary hypertension related to chronic lung diseases [50].

CLINICAL DATA

In addition to the previously described in vitro data and animal experiments, the antifibrotic effects of treprostinil have been brought to light through clinical studies as well. A post hoc analysis of the INCREASE study assessed FVC for the overall study population and subgroups, including patients with idiopathic interstitial pneumonia (IIP) and IPF [16]. Inhaled treprostinil treatment resulted in an overall FVC improvement of 28.5 mL ($P = 0.35$) and 44.4 mL ($P = 0.21$) at weeks 8 and 16, respectively, compared to placebo. Greater differences in FVC were seen in subgroups of patients with IIP (108.2 mL, $P = 0.0229$) and IPF (168.5 mL, $P = 0.0108$) at week 16. These larger numeric differences in FVC in the subgroups of patients with IIP and IPF compared to placebo are likely due to the IIP and IPF populations having a greater propensity for progression [16].

A question that these results raise is whether this difference in the FVC was due to an improvement of vascular compliance or if it indicates a true antifibrotic effect. To shed further light on this issue, a comparison of the FVC changes in the INCREASE and TRIUMPH studies was performed. The TRIUMPH study evaluated inhaled treprostinil over 12 weeks in patients with PAH [51]. Pulmonary function tests (PFTs) were conducted at baseline and week 12 in

TRIUMPH and percentage change in predicted FVC and forced expiratory volume in 1 s (FEV1) were evaluated using analysis of covariance (ANCOVA). In INCREASE, PFTs were completed at baseline and weeks 8 and 16. The mixed model repeated measurement was used to evaluate the change in percentage predicted FVC and FEV1 [52]. This post hoc analysis found that PFT response to inhaled treprostinil differs between PAH and PH-ILD as improvements in percentage predicted FVC were seen in patients with PH-ILD but not in those with PAH [52]. These differences support treprostinil's antifibrotic mechanism of action, suggesting amelioration in the fibrotic lung disease rather than through its effects on the pulmonary vasculature, further promoting the potential therapeutic role of treprostinil for patients with PH-ILD.

CONCLUSION

Treprostinil's pharmacological profile via its ability to activate IP, DP_1, and EP_2 receptors, coupled with the robust expression of the last two receptors in PAH, suggests that its use could provide additional benefits as an antifibrotic agent. Inhibition of both TGFβ1 and PDGF is unique to treprostinil as other approved therapies for ILD only affected either TGFβ1 or PDGF [53, 54].

Treprostinil also had a significant effect on PPARs. PPARβ and PPARγ both demonstrated antiproliferative effects on human lung fibroblasts with in vitro treprostinil treatment.

Preclinical and animal model research supports that treprostinil may exert its therapeutic effect via multiple pathways, including inhibiting fibroblast-to-myofibroblast differentiation, fibroblast proliferation [33, 44, 49, 55], fibroblast migration [32, 39], fibronectin deposition [36], and mast cell-mediator release [56].

The in vitro and in vivo evidence of treprostinil's antiproliferative potential is intriguing evidence for its therapeutic potential as an inhaled treatment for lung fibrosis. Treprostinil is unique among the prostacyclin mimetics approved for treating PAH in that it has distinct actions at additional prostaglandin receptors that contribute to its therapeutic benefit.

There is yet to be an inhaled therapy with proven antifibrotic effects. Patients with IPF may benefit from an inhaled therapy owing to targeted deposition of drug at the disease site, rapid onset of action, and fewer systemic side effects than are seen with oral administration. In addition, with fibrosis causing lung architectural distortion and associated vascular ablation, it is uncertain where in the lung any systemically delivered agent is actually deposited and if deposition is to the target areas of activity to facilitate amelioration of the fibrosis.

In summary, this overview provides a sound biologic and physiologic basis for a clinical trial which is currently underway to assess the efficacy of inhaled treprostinil in patients with pulmonary fibrosis (NCT04708782) [57].

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