Viewpoint

Could a Nebulized or Dry Powder Inhalation of a Dopamine D1R Agonist Be a Treatment of Idiopathic Pulmonary Fibrosis?

Idiopathic pulmonary fibrosis (IPF) is a rare, debilitating disease resulting in the growth of fibroblasts and collagen deposition within the alveolar sac, which at best diminishes and at worst eliminates oxygen transfer to the blood (Raghu et al., 2006). Given the current world population and IPF incidence estimates, approximately 71,000 to 1,032,000 people are diagnosed with IPF each year (Maher et al., 2021). Although two approved therapies exist, no treatment halts or reverses the fibrosis. Moreover, with treatment, lung function, as measured by forced vital capacity, which measures the total air that can be exhaled, continues to decline during therapy. The two therapies are nintedanib and pirfenidone. Nintedanib is a pan-tyrosine kinase inhibitor targeting multiple cytosolic tyrosine kinases and receptor tyrosine kinases involved in cellular proliferation and migration, and the mechanism of action of pirfenidone is not entirely elucidated, yet it suppresses endogenous transforming growth factor beta 1 (TGF-β1) production. TGF-β1 is thought to be a significant driving force underlying IPF (Kim et al., 2006).

It is accepted that TGF-β-mediated signaling leads to YAP/TAZ activation (Pefani et al., 2016). YAP/TAZ is involved in collagen synthesis and promotes epithelial-mesenchymal transition (Lei et al., 2008; Zhao et al., 2008; Jorgenson et al., 2017; Kegelman et al., 2018); therefore, inhibiting YAP/TAZ should reduce TGF-β-mediated fibrosis. Gzα-mediated elevations of cAMP lead to protein kinase A activation of LATS1/2, and subsequent YAP/TAZ phosphorylation and cytosolic retention (Yu et al., 2013). Since YAP/TAZ translocates to the nucleus and coactivates the transcription factor TEAD, retaining YAP/TAZ in the cytosol inhibits YAP/TAZ function. Thus, it is unsurprising that cAMP can prevent TGF-β1-mediated collagen synthesis (Liu et al., 2006).

In this issue, Gao and colleagues begin by examining a subset of RNA sequencing (RNAseq) data from 2019 that included vehicle control and TGF-β1-treated fibroblasts from three IPF patients. The RNAseq data indicated that TGF-β1 reduced the expression of nearly all Gzα-coupled G protein coupled receptors, with the β2-adrenergic receptor (β2-AR) demonstrating the greatest decrease. However, the D1 dopamine receptor (D1DR) and the orphan G protein coupled receptor 3 are spared. Gao et al. then transition to using primary human lung fibroblasts purchased from Lonza to determine if the RNAseq data can be replicated. TGF-β1 nearly eliminates β2-AR mRNA and slightly increases D1DR mRNA through an activin receptor-like kinase-dependent mechanism. Functionally, D1DR-signaling is not altered by TGF-β1, whereas β2-AR-mediated signaling is ablated. The paper by Gao et al. continues similarly to previous work from the Haak laboratory, demonstrating that D1DR signaling reduces YAP/TAZ activity, genes involved in fibrosis, and deposited collagen (Haak et al., 2019; Diaz-Espinosa et al., 2020; Choi et al., 2021). The D1DR-mediated effects are expected, given the effects of cAMP on YAP/TAZ and TGF-β1-mediated collagen synthesis (Liu et al., 2006; Yu et al., 2013).

The strength of the manuscript by Gao et al. is primarily demonstrating that the D1DR is functionally spared from TGF-β1-mediated downregulation via demonstrating D1DR full agonists dihydrexidine and A-77636 mediated effects when β-adrenergic receptor agonists have no effect. However, the apparent increase in D1DR fails to change D1DR signaling from full and partial agonists. Previous studies demonstrated that TGF-β1-mediated epithelial-mesenchymal transition of alveolar type II cells leads to an increase in the cAMP-specific phosphodiesterase 4 (PDE4) (Kolosionek et al., 2009); similarly, in tracheal smooth muscle, TGF-β1 reduces Gzα-mediated signaling (Chung et al., 2020). As PDE4 was not examined, it is unclear if the increase in receptor signaling is buffered by an increase in PDE4. Although Gao et al. suggest that increased D1DR mRNA could increase spare receptors, it is more likely that the increased receptor level is not biologically significant. Gao et al. demonstrated that after 72 hours in culture, the control group demon-
strated increased D1DR mRNA to a degree similar to TGF-β1 treatment. Moreover, the total increase in D1DR mRNA is below or near the traditional twofold change cutoff. Further studies are required to fully understand the details underlying D1DR-mediated cAMP signaling in the presence of TGF-β1; yet, regardless of the details, retaining normal D1DR-mediated cAMP production in the presence of TGF-β1 is enough to entertain the idea that D1DR agonists can act as a novel IPF therapy.

The manuscript has two minor weaknesses that deserve discussion. Foremost, the model of treating fibroblasts with TGF-β1 does not fully represent pathologic fibroblasts from patients. Given that the group was part of the 2019 study that used IPF patient fibroblasts and that Lonza sells lung fibroblasts isolated from donors with IPF, it is surprising that diseased cells were not used in conjunction with the normal human lung fibroblasts. Adding diseased cells would strengthen the conclusions and, if the D1DR expression remains, more strongly support the idea that D1DR agonists can act as a novel IPF therapy. Granted, the group has shown reversal of fibrosis in vivo following stimulation of the D1DR with internasal delivery of dihydrexidine (Haak et al., 2019); therefore, this weakness is blunted by the group’s previous findings and suggests that readers of Gao et al. should read the group’s previous publications as well.

The second minor weakness is that the nuclear versus cytosolic localization of YAP/TAZ presented by Gao et al. is conducted only via epifluorescence imaging. Thus the YAP/TAZ localization is not the most robust presentation. A second assay supporting the epifluorescence imaging, such as demonstrating YAP/TAZ phosphorylation, would enhance the YAP/TAZ location interpretation. Fortunately, Gao et al. conducted a TEAD-luciferase reporter assay, as displayed in their supplemental material, demonstrating that dihydrexidine and A-77636 inhibit TEAD activity, which supports the localization data; however, the data are incomplete without the addition of the D1DR partial agonists and β2-AR agonists. The Haak group previously conducted YAP/TAZ nuclear and cytosolic fractionation, YAP/TAZ phosphorylation, and epifluorescence imaging, demonstrating that D1DR-mediated cAMP signaling can inhibit YAP/TAZ (Choi et al., 2021). Thus, as with the previous weakness, the Gao et al. manuscript is bolstered by the group’s previous publications; however, this also diminishes the novelty of the second half of the manuscript.

Increasing cAMP through the D1DR is an intriguing prospect to treat and perhaps revert pulmonary fibrosis to normal tissue (Haak et al., 2019). Can a D1DR agonist be nebulized or produced as a dry powder for inhalation for delivery to human lungs, or is it better to take a D1DR agonist orally to treat IPF? If directly targeting the lung, is it important for the ligand to be retained within the lung due to low absorption like the quaternary ammonium salt muscarinic antagonists, or is it better to have a lipophilic drug that is more likely to reach sites of fibrosis within the interstitium? As already conducted by the Haak group with dihydrexidine (LogP 2.5), the bleomycin model of IPF can be used to test various inhaled and orally delivered D1DR agonists. For example, A-68930, a partial D1DR agonist used by Gao et al., has the lowest predicted LogP (1.49) of all D1DR-specific agonists, suggesting it is the least lipophilic and may be retained within the lung, reducing systemic exposure. Additionally, A-68930 was not statistically different from dihydrexidine in reducing YAP/TAZ nuclear localization after plating pulmonary fibroblasts on plastic (n = 4, t-test P = 0.499), nor reducing TGF-β1-mediated z-smooth muscle actin expression in pulmonary fibroblasts (n = 4, t-test P = 0.352) (data analyzed from the supplementary files from the dopaminergic ligand library screen in Haak et al., 2019). Alternatively, SKF-83822 has the greatest predicted LogP (4.44) and may easily permeate the entire lung, including the interstitium where the pulmonary fibroblasts reside; SKF-83822 was not part of the dopaminergic ligand library. Therefore, these two D1DR agonists, plus clinically viable agonists, can be used to determine what pharmacoechemical properties of D1DR ligands and delivery modes are best for treating IPF.

If the lung can be precisely targeted, there would be less systemic exposure, thereby reducing unwanted neuronal and cardiovascular effects. Ideally, a locally delivered D1DR agonist would improve pulmonary fibrosis with minimal systemic effects. There are many clinically approved D1DR agonists; one may be able to be nebulized or made into a dry powder for inhalation and used as an experimental treatment of IPF.

Although a rare disease, approximately 263,000 to 3,589,000 people are afflicted with IPF worldwide (Maher et al., 2021). Current therapies slow the decline in forced vital capacity but do not halt or reverse pulmonary fibrosis; thus, there is a need for novel therapies to treat IPF patients. The manuscript by Gao et al., in conjunction with the group’s previous publications, convincingly demonstrates that the D1DR is a viable pharmacological target for treating IPF. Given that there are clinically viable D1DR agonists, it is not a stretch to hope that an IPF treatment that can potentially halt and resolve fibrosis will soon be developed.

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Data Availability
This article contains no datasets generated during the current study. The statistical analysis was conducted on data sets from the supplementary files within Haak et al., 2019.

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References