1521-0103/386/3/274–276$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

dx.doi.org/10.1124/jpet.123.001661
J Pharmacol Exp Ther 386:274–276, September 2023

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. Gα_s-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 Gα_s-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 Gα_s-mediated signaling (Chung et al., 2020). As PDE4 was not examined, it is unclear if the increase in receptor signaling is buffeted 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-

Address correspondence to: Dr. Bradley T. Andresen, FAHA, Western University of Health Sciences, College of Pharmacy, Pharmaceutical Sciences Department, 309 E. Second St., Pomona, CA 91766-1854. E-mail: bandresen@westernu.edu
No author has an actual or perceived conflict of interest with the contents of this article.
dx.doi.org/10.1124/jpet.123.001661.

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/YAZ 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 α-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 pharmacochemical 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.

Bradley T. Andresen

Department of Biotechnology and Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, Pomona, California

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.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Andresen.

References

Choi KM, Haak AJ, Diaz Espinosa AM, Cummins KA, Link PA, Aravamudhan A, Wood DK, and Tschumperlin DJ (2021) GPCR-mediated YAP/TAZ inactivation in fibroblasts via EPAC1/2, RAP2C, and MAP4K7. *J Cell Physiol* **236:**7759–7774.

Chung E, Ojiaku CA, Cao G, Parikh V, Deeney B, Xu S, Wang S, Panettieri Jr RA, and Koziol-White C (2020) Dexamethasone rescues TGF-β1-mediated β_2-adrenergic receptor dysfunction and attenuates phosphodiesterase 4D expression in human airway smooth muscle cells. *Respir Res* **21:**256.

Diaz-Espinosa AM, Link PA, Sicard D, Jorba I, Tschumperlin DJ, and Haak AJ (2020) Dopamine D1 receptor stimulates cathepsin K-dependent degradation and resorption of collagen I in lung fibroblasts. *J Cell Sci* **133:**jcs248278.

Gao AY, Diaz Espinosa AM, Nguyen BBN, Link PA, Meridew J, Jones DL, Gibbard DF, Tschumperlin DJ, and Haak AJ (2023) DRD1 is exempt from TGFß-mediated antifibrotic GPCR landscape tampering in lung fibroblasts. *J Pharmacol Exp Ther* **386:**277–287.

Haak AJ, Kostallari E, Sicard D, Ligresti G, Choi KM, Caporarello N, Jones DL, Tan Q, Meridew J, Diaz Espinosa AM et al. (2019) Selective YAP/TAZ inhibition in fibroblasts via dopamine receptor D1 agonism reverses fibrosis. *Sci Transl Med* **11:**eauu6296.

Jorgenson AJ, Choi KM, Sicard D, Smith KM, Hiemer SE, Varelas X, and Tschumperlin DJ (2017) TAZ activation drives fibroblast spheroid growth, expression of profibrotic paracrine signals, and context-dependent ECM gene expression. *Am J Physiol Cell Physiol* **312:**C277–C285.

Kegelman CD, Mason DE, Dawahare JH, Horan DJ, Vigil GD, Howard SS, Robling AG, Bellido TM, and Boerckel JD (2018) Skeletal cell YAP and TAZ combinatorially promote bone development. *FASEB J* **32:**2706–2721.

Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, and Chapman HA (2006) Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. *Proc Natl Acad Sci USA* **103:**13180–13185.

Kolosionek E, Savai R, Ghofrani HA, Weissmann N, Guenther A, Grimminger F, Seeger W, Banat GA, Schermuly RT, and Pullamsetti SS (2009) Expression and activity of phosphodiesterase isoforms during epithelial mesenchymal transition: the role of phosphodiesterase 4. *Mol Biol Cell* **20:**4751–4765.

Lei QY, Zhang H, Zhao B, Zha ZY, Bai F, Pei XH, Zhao S, Xiong Y, and Guan KL (2008) TAZ promotes cell proliferation and epithelial-mesenchymal transition and is inhibited by the hippo pathway. *Mol Cell Biol* **28:**2426–2436.

Liu X, Sun SQ, Hassid A, and Ostrom RS (2006) cAMP inhibits transforming growth factor-beta-stimulated collagen synthesis via inhibition of extracellular signal-regulated kinase 1/2 and Smad signaling in cardiac fibroblasts. *Mol Pharmacol* **70:**1992–2003.

Maher TM, Bendstrup E, Dron L, Langley J, Smith G, Khalid JM, Patel H, and Kreuter M (2021) Global incidence and prevalence of idiopathic pulmonary fibrosis. *Respir Res* **22:**197.

Pefani DE, Pankova D, Abraham AG, Grawenda AM, Vlahov N, Scrace S, and O' Neill E (2016) TGF-β targets the Hippo pathway scaffold RASSF1A to facilitate YAP/SMAD2 nuclear translocation. *Mol Cell* **63:**156–166.

Raghu G, Weycker D, Edelsberg J, Bradford WZ, and Oster G (2006) Incidence and prevalence of idiopathic pulmonary fibrosis. *Am J Respir Crit Care Med* **174:**810–816.

Yu FX, Zhang Y, Park HW, Jewell JL, Chen Q, Deng Y, Pan D, Taylor SS, Lai ZC, and Guan KL (2013) Protein kinase A activates the Hippo pathway to modulate cell proliferation and differentiation. *Genes Dev* **27:**1223–1232.

Zhao B, Ye X, Yu J, Li L, Li W, Li S, Yu J, Lin JD, Wang CY, Chinnaiyan AM et al. (2008) TEAD mediates YAP-dependent gene induction and growth control. *Genes Dev* **22:**1962–1971.