Treprostinil reduces clinically relevant fibrosis biomarkers in the Scar-in-a-Jar model using a fibrotic cocktail

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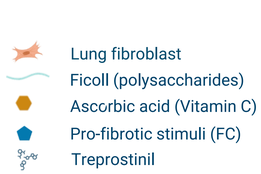
Background and aim

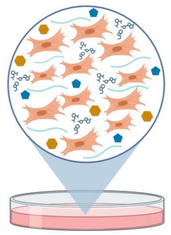
- The activation of fibroblasts and the subsequent deposition of extracellular matrix (ECM) play a pivotal role in the development of interstitial lung disease (ILD).
- Inhibiting fibrogenesis and extracellular matrix (ECM) deposition is crucial for novel anti-fibrotic drugs.
- In this study, we investigated the effects of a fibrotic cocktail (FC) that consists of eight pro-fibrotic and pro-inflammatory cytokines and one growth factor in the Scar-in-a-Jar fibroblast model, thereby mimicking the complex microenvironment associated with fibrotic ILDs.
- Treprostinil is approved for treatment of pulmonary arterial hypertension (PAH) and pulmonary hypertension in ILD (PH-ILD), and currently undergoing phase 3 trial for treatment of idiopathic pulmonary fibrosis (IPF).

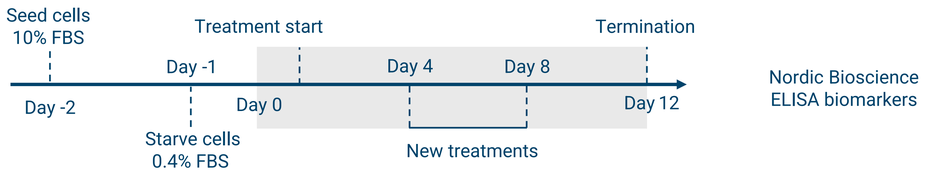
Aim: To evaluate the anti-fibrotic effect of Treprostinil in the Scar-in-a-Jar fibrogenesis model using human lung fibroblasts stimulated with a fibrotic cocktail (FC).

Methods

Scar-in-a-Jar







- Healthy human primary lung fibroblasts were cultured for 12 days in low-serum media supplemented with polysaccharides (Ficoll) and ascorbic acid (Vitamin C) to promote a macromolecular environment and efficient collagen folding[1] .
- Fibroblasts were incubated with pro-fibrotic stimuli (FC) with or without clinically relevant concentrations of Treprostinil[3] or vehicle (0.03% DMSO).
- Supernatant from fibroblast cultures was collected on day 4, 8 and 12 and appropriate treatments were renewed on day 4 and 8.
- Biomarkers nordicPRO-C3™, nordicPRO-C6™ and nordicFBN-C™ were quantified in the supernatant using Nordic Bioscience ELISAs.

Fibrotic cocktail (FC)

The composition of the FC is based on findings from bronchoalveolar lavage fluid collection from IPF patients[2] and consists of eight cytokines and one growth factor.

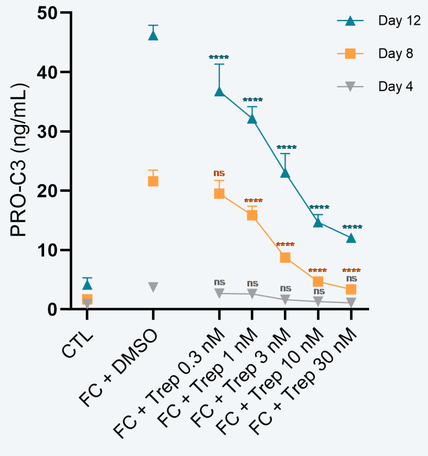
Fibrotic cocktail	[ng/mL]
TGF-β1	0.3
IL-1β	0.01
TNF-α	0.1
IL-8	1.5
MCP1 (CCL2)	0.7
IL-33	0.04
TSLP	0.1
IL-13	2.5
IL-4	0.16

[1]Rønnow et al. Resp. Research 2020. [2]Schruf et al. FASEB J. 2020
[3] Kumar et al. Clin Pharmacokinet. 2016

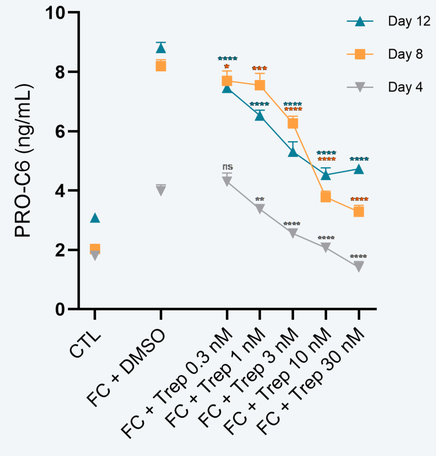
Results

Fibrosis biomarkers – PRO-C3, PRO-C6 and FBN-C

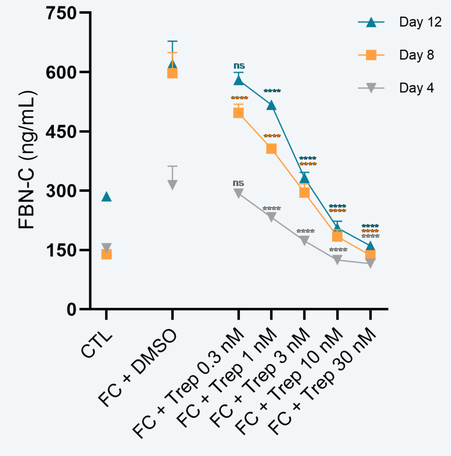
Type III collagen



Type VI collagen



Fibronectin



Fibrosis biomarkers quantified in supernatants from the Scar-in-a-Jar model. CTL: Control without stimulation, FC: Fibrotic cocktail, Trep: Treprostinil. Data are shown as mean ± SD of 4 replicates per treatment and analyzed using two-way ANOVA with multiple comparison test. * Indicates significant difference compared to vehicle (FC + DMSO) at day 4, 8 and 12, α < 0.05. **** < 0.0001. ns: not significant.

Conclusion

- The FC effectively stimulated fibrogenesis in the Scar-in-a-Jar in-vitro model, leading to elevated levels of the fibrosis biomarkers nordicPRO-C3™, nordicPRO-C6™ and nordicFBN-C™.
- Treprostinil significantly inhibited fibrogenesis and collagen deposition quantified by ELISA.
- The Scar-in-a-Jar model is a useful tool for screening novel anti-fibrotic drugs.

NORDIC BIOSCIENCE

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Disclosures: All authors are employed at Nordic Bioscience and may be shareholders.