Treating inflammation in respiratory diseases by inhaled aryl hydrocarbon receptor ligands and activators

M. Puccetti[1], M. Pariano[2], D. Traini[3], W. Paulina[1], A. Schoubben[1], M. Ricci[1] & S. Giovagnoli[1]

[1]Department of Pharmaceutical Science, University of Perugia, Perugia,06123,Italy

[2]Department of Medicine and Surgery, University of Perugia, Perugia, 06132, Italy

[3]Macquarie Medical School, Faculty of Medicine, Health, and Human Sciences, Macquarie University, Sydney, NSW 2109, & The Woolcock Institute of Medical Research, Sydney, NSW, 2031, Australia

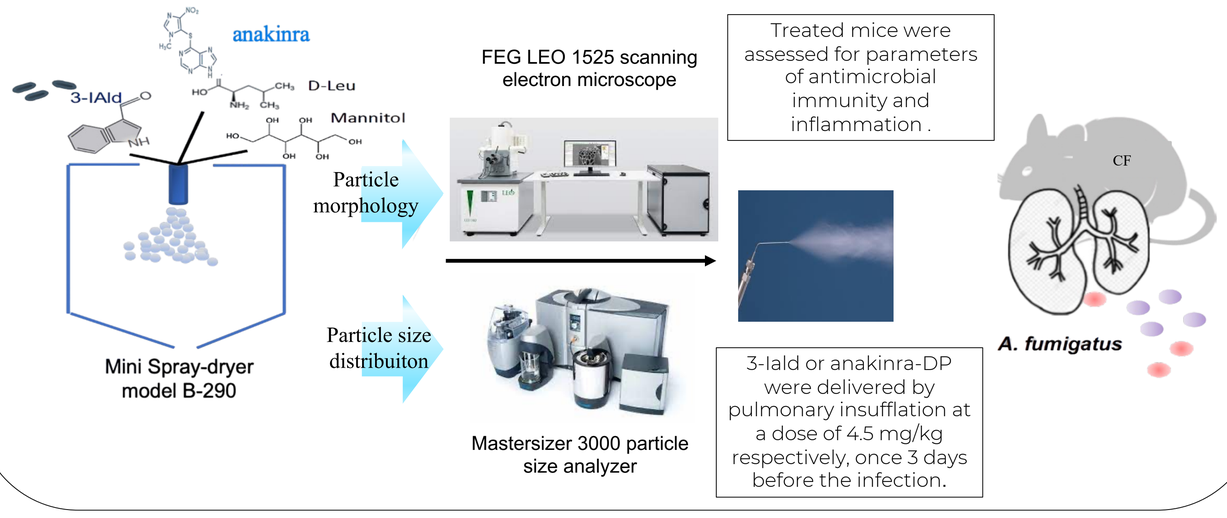
Background

Due to mechanical, chemical and immunological barriers to the respiratory tract, pulmonary drug delivery is complex and demands strategies and formulations that maximize airway selectivity. The association between inflammation and chronic disease is still under investigation and how to translate the body of knowledge into effective strategies for the prevention and treatment of pathologic inflammation is lagging. In this regard, in this study, a dry powder formulation acting on the xenobiotic aryl hydrocarbon receptor (AhR) to regulate lung function and inflammation is presented and in vivo pharmacological properties and toxicity are assessed. Targeting of AhR is of great medical and pharmaceutical interest for inhaled therapies targeting diseases in which immunopathology plays a pathogenetic role. However, despite the availability of several structurally diverse ligands of AhR, the bottleneck in targeting this receptor is its ubiquitous expression and its functional activity that is both contexts and ligand-dependent.

Aims

In this study, we provide a proof-of-concept demonstration of the druggability of AhR in lung inflammation via inhalable dry powders of either the tryptophan metabolite of microbial origin, indole-3-aldehyde, an AhR ligand [1], or of the recombinant form of the endogenous IL-1 receptor antagonist (anakinra), an AhR modulator. Either immunomodulatory treatment resolved inflammation without compromising the ability of the immune system to respond to pathogens and with no signs of unwanted toxicities and off-target effects.

Methods



Results

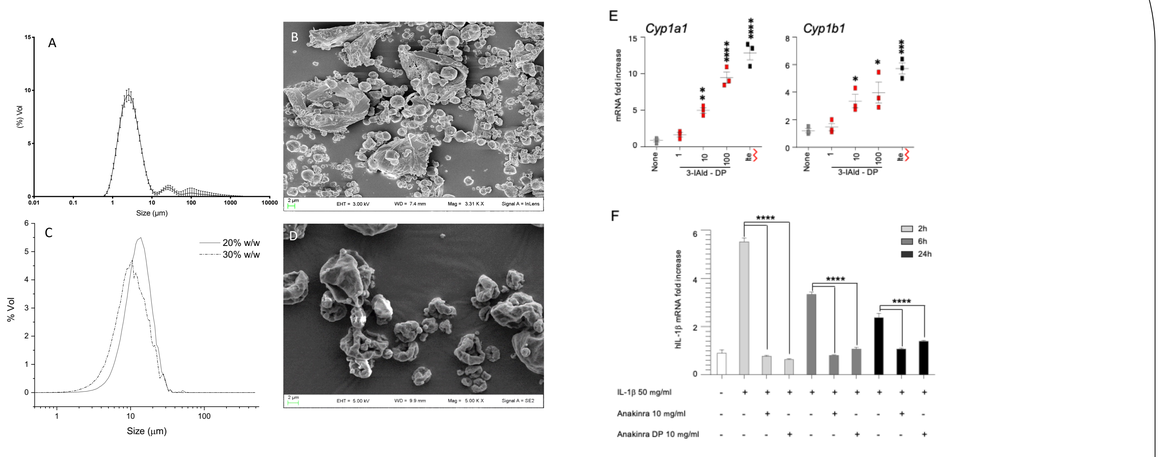


Figure 1 - A) Particle size distribution of 3-IAld dry powder (Mannitol:3-IAld ratio of 2:1). B) SEM images of 3-IAld dry powder. C) Particle size distribution of Anakinra dry particle, loaded at 20 %w/w, showed a narrow population. D) SEM of anakinra dry powder

- The SEMs and particle size results showed 3-IAld (Figure 1 A, B) and anakinra (Figure 1 C, D) dry powders (DP) have a corrugated morphology with some agglomeration, with Anakinra, in particular, showing a typical irregular buckled shape. The aerodynamic particle size range was 3.7 ± 0.4 µm for 3-IAld formulation and 6 ± 0.2 for Anakinra formulation, respectively, suitable for inhalation drug delivery [2].
- We first showed that both 3-IAld-DP and anakinra-DP retained their pharmacological activity in vitro, as shown by the ability of 3-IAld-MP to activate the AhR-dependent Cyp1a1 and Cyp1b1 genes in the A549 lung epithelial cell line (C) and of anakinra-DP to inhibit IL-1beta-dependent activation of myeloid THP1 cells (D).

Results

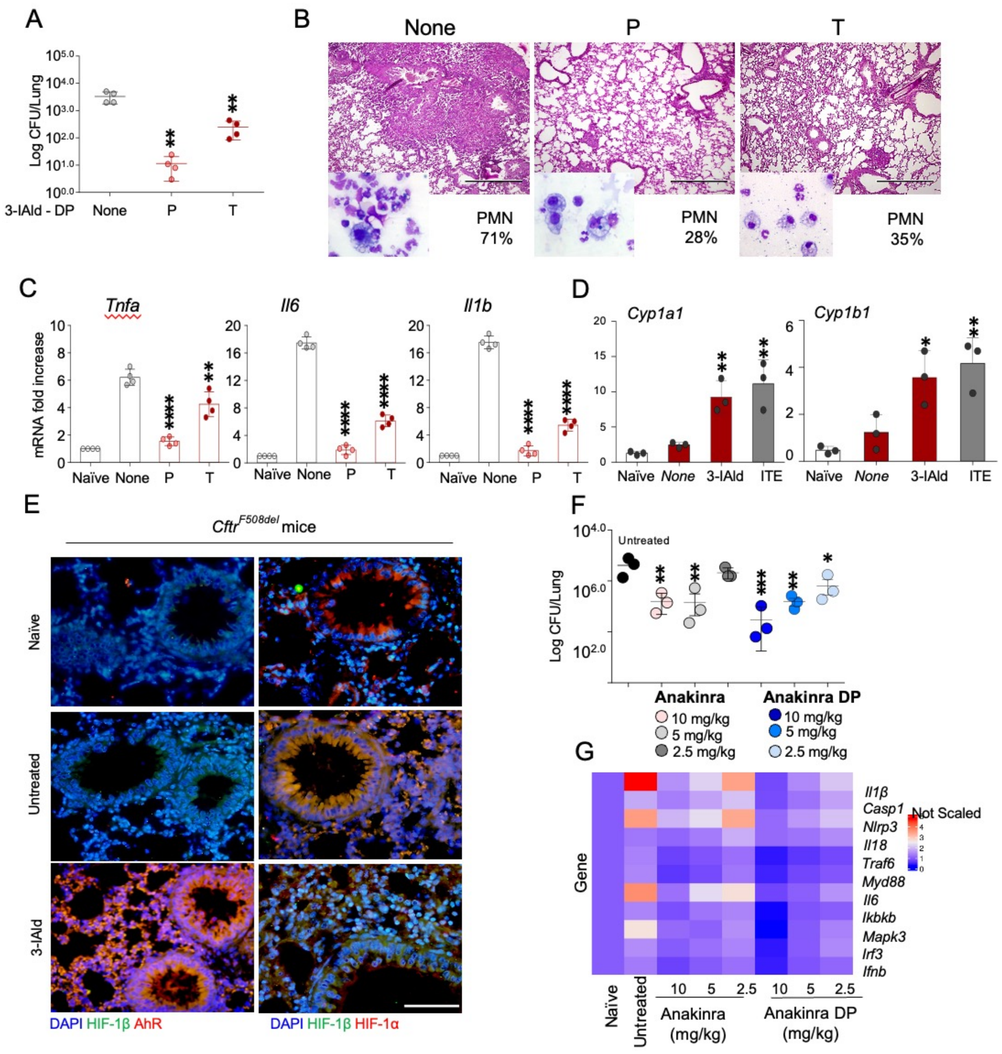


Figure 2. Fungal growth, lung hystophatology and inflammatory cytokine response in mice with Cystic Fibrosis infected with *Aspergillus fumigatus* and treated with 3-IAld-DP or anakinra-DP.

- We then evaluated the effects of either DP formulation on a murine model of Cystic fibrosis (CF), for its known lung inflammatory pathology to which HIF-1a-mediated hypoxia contributes. 3-IAld-DP (18 mg/kg) and anakinra-DP (different doses) were directly delivered into the lung 3 days after the infection with *Aspergillus fumigatus*. We found (Figure 2) that 3-IAld, given before (P) or after (T) the infection, greatly inhibited the fungal growth (A, expressed as Log10 CFU, colony-forming unit), the inflammatory pathology in the lung (B, by PAS staining), reduced neutrophil recruitment (% in the insets), decreased the expression of inflammatory cytokine genes (C) and activated the AhR-dependent genes (D, by RT-PCR). Of interest, given the interferential crosstalk between the AhR and HIF-1α signaling pathways, via the sharing of HIF-1β for their activation, 3-IAld also squelched the HIF-1α response, as observed by the immunofluorescence staining in the lung (E) [3]. Likewise, anakinra-DP, more than standard anakinra, dose-dependently inhibited fungal growth (F) and inflammatory gene (G) expression in the lung [4].

Conclusions

This study shows how dry powder formulation could be used to target AhR in the lung for immunomodulatory treatment, to resolve inflammation without compromising the ability of the immune system to respond to pathogens and with no signs of unwanted toxicities. Developing therapeutics for inflammatory diseases is challenging due to physiological mucosal barriers, systemic side effects, and the local microbiota. In the search for novel methods to overcome some of these problems, drug delivery systems that improve tissue-targeted drug delivery are highly desirable. We have shown that deciphering of how a signaling molecules, like 3-IAld, interacts with its targets paves the way for targeted therapies of inflammatory human diseases for the realization of which drug delivery strategies are instrumental.

Reference

1. Zelante, T., et al., Regulation of host physiology and immunity by microbial indole-3-aldehyde. Curr Opin Immunol, 2021. 70: p. 27-32.
2. Puccetti et al., Targeted Drug Delivery Technologies Potentiate the Overall Therapeutic Efficacy of an Indole Derivative in a Mouse Cystic Fibrosis Setting. Cells, 2021. 10(7).
3. Pariano et al, Aryl hydrocarbon receptor agonism antagonizes the hypoxia-driven inflammation in Cystic Fibrosis. Am J Respir Cell Mol Biol. 2022 Oct 17. doi: 10.1165/rcmb.2022-0196OC
4. Puccetti M et al. Pulmonary drug delivery technology enables anakinra repurposing in cystic fibrosis. JCR, manuscript revised.

Supported by the FFC#17/2020