Fenretinide prevents the development of age-related lung disease in CFTR-knockout mice and overproduction of MUC5AC

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Introduction: Cystic fibrosis (CF) is the most common genetic disease in Caucasians, caused by different mutations in the CFTR gene. Hyperproduction of mucus, gradual decline in forced expiratory volume (FEV1) and persistent hyperinflammation have long been recognized as the age-related pathologies of CF. Fenretinide (FEN), a derivative of vitamin A, has been shown to revert the pro-inflammatory imbalance of fatty acids and ceramides in CF mice and to inhibit the NF-κB pathway in cancer cell lines and macrophages. Lung infection with *P. aeruginosa* induces the production of both MUC5AC and MUC5B mucins in the lungs. While excessive production of MUC5AC leads to pathological plugging of the airways, the MUC5B gene was shown to be essential for clearing bacterial infections.

Methods: We used the CFTR knockout mice on a C57Bl/6 genetic background (CFTR-KO) as a model to study the progression of CF lung disease. We measured airway resistance in response to increasing concentration of methacholine, an experimental parameter in mice that most closely correlates with human FEV1. Older (7 months) mice were treated for 21 days with 10 mg/kg of fenretinide (Lau-7b). As a model of chronic infection, mice were infected with *P. aeruginosa* (PA508) embedded in agarose beads. For histological assessment, lungs were stained with Periodic Acid–Schiff/Alcian Blue to evaluate mucus production and Hematoxylin/Eosin (H&E) to evaluate the infiltration of inflammatory cells. We have used SPOC-1 lung goblet cells to analyze mucus production induced by bacterial lipopolysaccharide (LPS). Gene expression was analyzed at the mRNA level by quantitative real-time PCR (qPCR) and at the protein level by ELISA assays.

Results: Our data demonstrates that young CFTR-KO mice do not differ from their wild-type littermates (WT) in the production of mucus at the baseline. Their production of mucus upon bacterial infection is similar to the WT. However, uninfected older CFTR-KO mice show a much higher degree of infiltration of inflammatory cells in the lungs than WT, which can be prevented by FEN treatment. We also show that CF-KO mice display higher methacholine-induced airway resistance than WT and that FEN treatment efficiently corrects this defect. Furthermore, we demonstrate for the first time that FEN treatment efficiently regulates the total production of mucus in the lungs of *P. aeruginosa* infected mice. Finally, FEN treatment also prevents the LPS-induced increase in MUC5AC expression, without significantly affecting the level of MUC5B gene expression in the SPOC-1 cell line.

Conclusion: Our data demonstrates age-dependent progression of CF lung disease in CFTR-KO mice. It also demonstrates the impaired lung physiology in CFTR-KO mice compared to their littermate control, which can be improved following FEN treatment. Overall, the protective effects of FEN treatment on CF lung disease and lung physiology as well as FEN-induced selective modulation of LPS-induced production of MUC5AC provide further support for the use of this therapy to prevent the progression of CF lung disease.

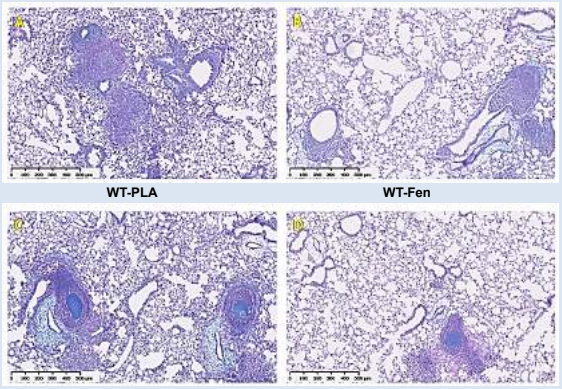

Figure 2. Pulmonary infection of mice with *P. aeruginosa*-coated agarose beads results in a massive infiltration of inflammatory cells and production of mucus in WT (A) and CFTR-KO (C). Fenretinide pre-treatment attenuates these effects in both WT (B) and CFTR-KO (D).

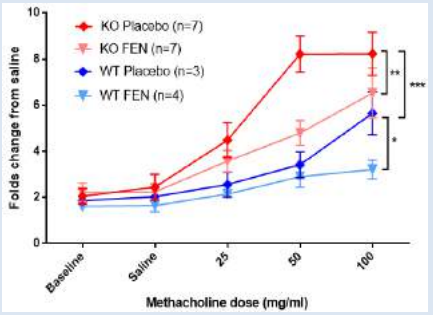

Figure 3. Airway resistance in response to escalating concentrations of methacholine is significantly higher in uninfected CFTR-KO mice than their WT littermates. Fenretinide treatment of both CFTR-KO and WT mice significantly decreases methacholine-induced lung resistance.

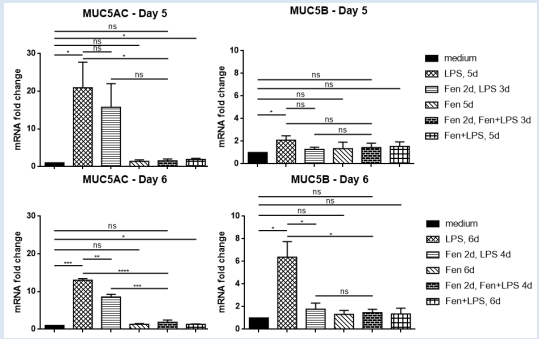

Figure 4. In a pulmonary goblet cell line (SPOC-1), LPS treatment strongly induces (~20 fold) transcription of mRNA for MUC5AC mRNA by day 5 and mRNA for MUC5B by day 6 (~6 fold). Pre-treatment of cells with fenretinide as well as combined treatment with fenretinide and LPS strongly attenuates LPS-induced transcription of mucin genes.

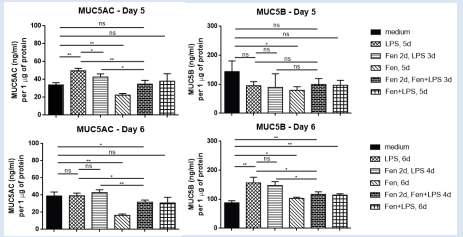

Figure 5. Protein levels of the MUC5AC gene follow the kinetics of mRNA induction by LPS in SPOC-1 cells, whereas the protein level of MUC5B gene is strongly induced only after day 6 of LPS treatment. Fenretinide pre-treatment as well as combined fenretinide+LPS treatment prevents LPS-induced increase in MUC5AC protein. The level of MUC5B protein is not significantly affected by fenretinide nor fenretinide+LPS treatment compared with the mock control, but the increase seen in the treatment with LPS alone is diminished by fenretinide treatment.

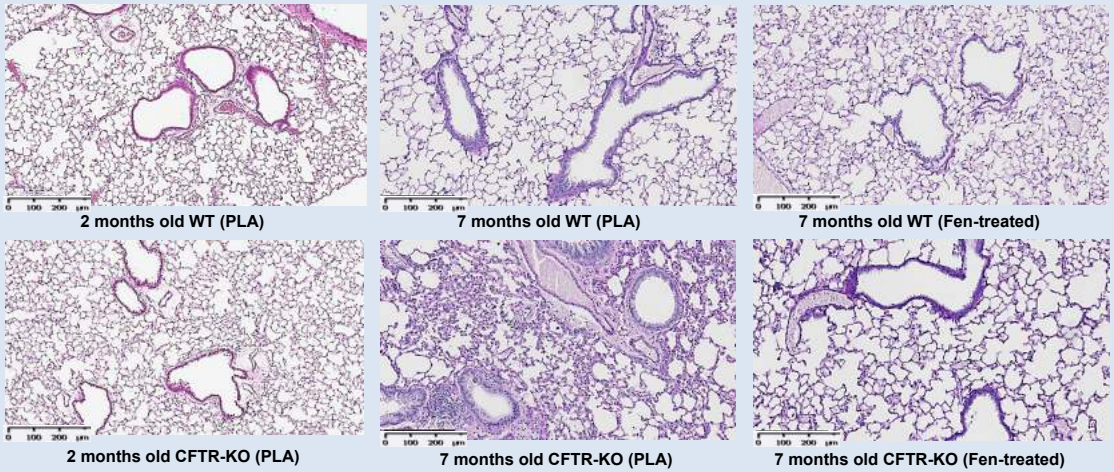

Figure 1. Young CFTR-KO mice (2 months) do not display a hyperinflammatory phenotype in the lungs that becomes apparent in older CFTR-KO mice (7 months), even in the absence of bacterial or viral infection. Fenretinide prevents the development of age-related lung hyperinflammation in older CFTR-KO mice.

Conclusions

- Fenretinide prevents the development of age-related pulmonary hyper-inflammation and epithelial hyperplasia in CFTR-KO mice.
- Fenretinide improves lung physiology by decreasing airway resistance in CFTR-KO mice.
- Fenretinide selectively inhibits the induction of MUC5AC gene expression in lung goblet cells, but has a very modest effect on MUC5B gene expression. Fenretinide inhibits endotoxin-mediated mucus accumulation in lungs.
- Fenretinide inhibits accumulation of mucus in the lungs of mice infected with *P. aeruginosa*.

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