Steroid-resistant inflammation in a mouse model of severe asthma is not inhibited by the combination of Theophylline and Budesonide

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Introduction

Severe asthma remains a major unmet medical need and results in a significant demand on healthcare costs. One of the key characteristics of the severe phenotype is a neutrophilic inflammation that is resistant to steroid treatment usually effective in the mild/moderate disease. Steroid-resistant neutrophilic inflammation is also a key characteristic of Chronic Obstructive Pulmonary Disease (COPD) where it has been demonstrated to be due to reduced HDAC activity which can be reversed by the combination of low dose Theophylline and steroid. We established two house dust mite (HDM) models of allergic asthma and demonstrated one (Alum/HDM) to model the steroid-sensitive eosinophilic inflammation associated with mild/moderate asthma and the other (Complete Freund's Adjuvant (CFA)/HDM) to model the steroid-resistant neutrophilic inflammation associated with severe asthma (Figures 1, 2, 3 and 4). We then investigated whether the steroid resistance of the severe asthma model could be reversed by using a combination of Theophylline and steroid previously shown to be effective in a mouse model of COPD (Fox JC et al, Proc. Am. Thorac. Soc., 2007).

Neutrophils, 24hr, HDM/CFA model

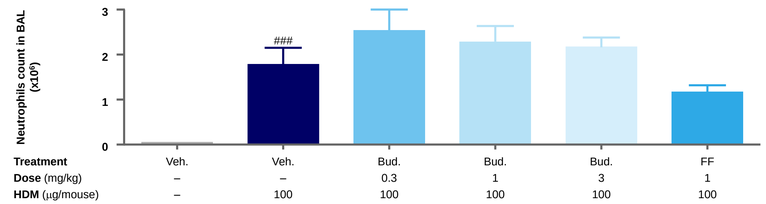

Figure 3. Neutrophilic inflammation is resistant to steroid treatment in the severe asthma model. n=8 per group, ###p<0.001, Student's t-test.

Methods

Mice were sensitised with HDM (100µg, s.c.) in Complete Freund's Adjuvant and were challenged with HDM (100µg, i.n.) 14 days later. Mice were pre-treated, 1 hour prior to challenge, with vehicle, Budesonide (1mg/kg, i.n.), Theophylline (1mg/kg, i.n.) or the combination of Budesonide and Theophylline. Lungs were lavaged 24 hours after challenge and neutrophil and eosinophil counts performed.

Results

HDM-challenge caused a significant increase in neutrophil and eosinophil counts in the lung lavage.
When tested alone, neither Theophylline nor Budesonide resulted in inhibition of neutrophilia.
The combination of Theophylline and Budesonide similarly failed to produce a statistically significant inhibition of the neutrophil response (Figure 6).
The increased BAL eosinophil counts were similarly unaffected (data not shown).

Neutrophils

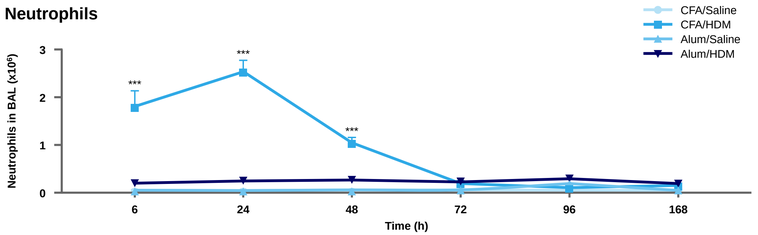

Figure 1. Timecourse of neutrophilic inflammation in severe and mild/moderate HDM asthma models in mice.
n=8 per group, ***p<0.001, Student's t-test.

Eosinophils, 48hr, HDM/Alum model

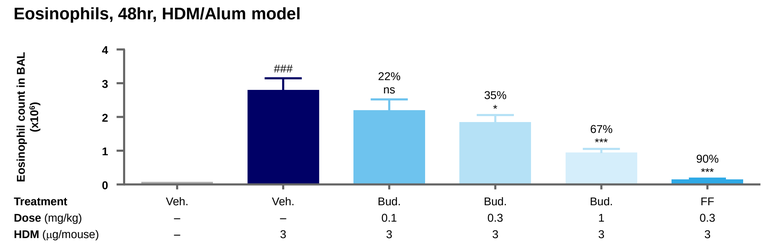

Figure 4. Eosinophilic inflammation is sensitive to steroid in the mild/moderate asthma model. n=8 per group, ### p<0.001, Student's t-test.
* p<0.05, *** p<0.001. Two-way ANOVA followed by Dunnett's test for multiple comparisons.

Neturophils, 24hr, CFA/HDM model

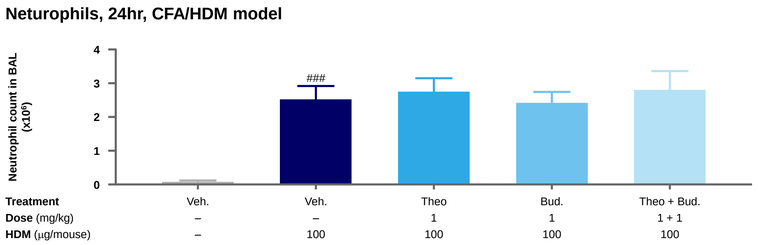

Figure 6. Effect of single and combination of intranasal dose of Theophylline and Budesonide on BAL neutrophilia.
n=8 per group, ### p<0.01, Student's t-test.

Eosinophils

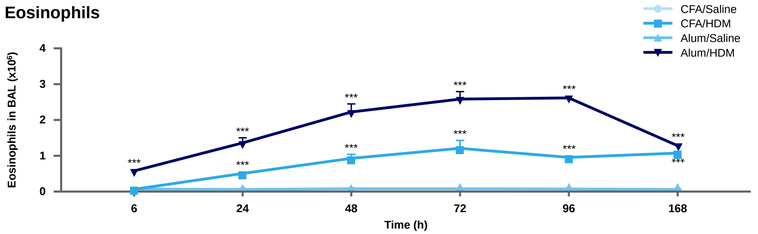

Figure 2. Timecourse of eosinophilic inflammation in severe and mild/moderate HDM asthma models in mice.
n=8 per group, ***p<0.001, Student's t-test.

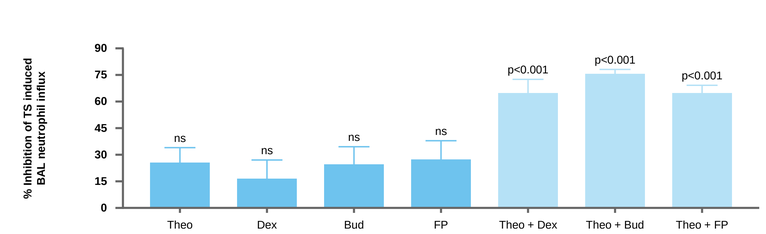

Figure 5. Combination of ineffective intranasal doses of Theophylline (0.5mg/kg) and either Dexamethasone, Budesonide or Fluticasone Proprionate (0.1mg/kg) inhibit BAL Neutrophilia in mice exposed to tobacco smoke for 11 days (Fox JC et al, Proc. Am. Thorac. Soc., 2007).

Conclusion

- Budesonide was ineffective against the neutrophilic inflammation caused by HDM sensitisation and challenge in the severe asthma model at a dose level shown to be effective in the mild/moderate asthma model. This mimics the human disease phenotype.
- The combination with Theophylline was also ineffective, unlike in the mouse model of COPD where the combination was shown to inhibit the steroid -resistant neutrophilic inflammation
- Thus we conclude that the mechanisms of steroid-resistance in the two models may not be the same and separate pre-clinical animal models are required in the search for new therapeutics to treat COPD and severe asthma