IL-13 decreases M3R expression in the human airway, reducing the effect of tiotropium on mucin production

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Background

IL-13 and neutrophil elastase (NE) play important roles in mucus secretion in asthma. Tiotropium, a long-acting muscarinic antagonist, is used for therapy of COPD and increasingly, for asthma therapy. We hypothesized that tiotropium would block the effects of IL-13 or NE on mucin (MUC5AC) expression in cultured human airway cells, and that this would be mediated through the muscarinic 3 receptor (M3R).

Objectives

In differentiated normal human bronchial epithelial (NHBE) cells:

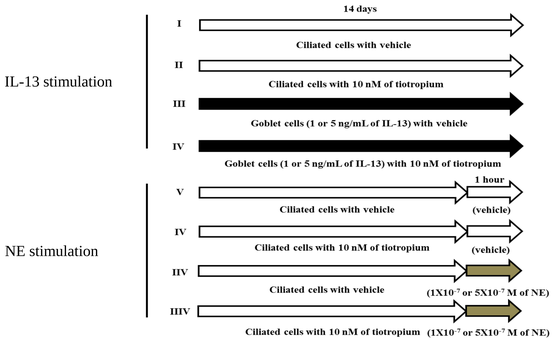
1. Evaluate the effect of tiotropium on IL-13-induced or NE-induced MUC5AC expression.
2. Assess the influence of IL-13 or NE on M3R expression.

Methods

Cell culture

NHBE cells were grown at air-liquid interface for 14 days with 0, 1, or 5 mg/mL IL-13. We exposed half of the wells in each group to 10 nM tiotropium or excipient for 14 days.

For NE stimulation, the cells were grown at air-liquid interface without IL-13, and then exposed to NE 1×10^{-7} or 5×10^{-7} M for 1 hour at day 14.

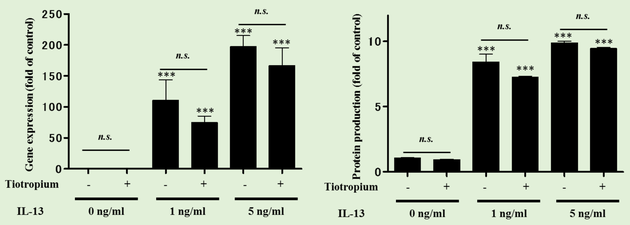


Measurements

MUC5AC was measured by quantitative PCR and ELISA. M3R expression was evaluated using quantitative PCR, Western blotting and immunohistochemistry.

Results

Figure 1. Tiotropium did not significantly decrease IL-13-induced MUC5AC mRNA expression and protein production.



***p<0.001 compared with control (N=3)

Figure 2. Tiotropium did not attenuate IL-13-induced goblet cell metaplasia.

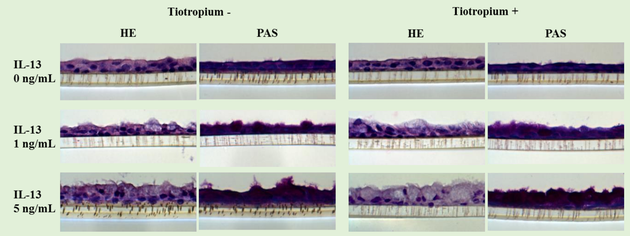
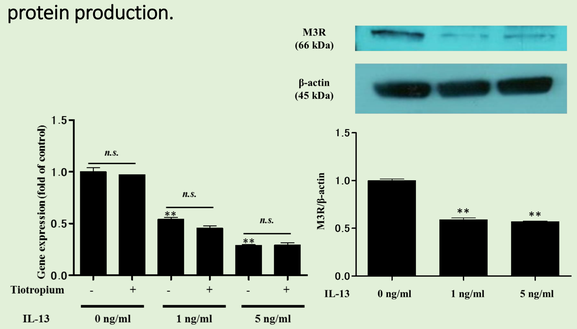


Figure 3. IL-13 significantly decreased M3R mRNA expression and protein production.



**p<0.01 compared with control (N=3)

Figure 4. Apical surface expression of M3R in IL-13 transformed goblet cells was less than that in ciliated cells.

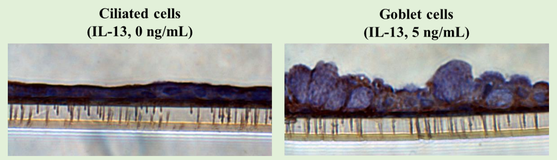
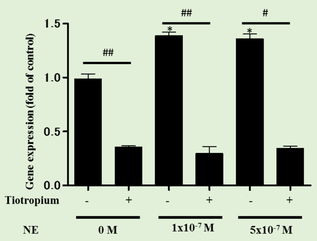
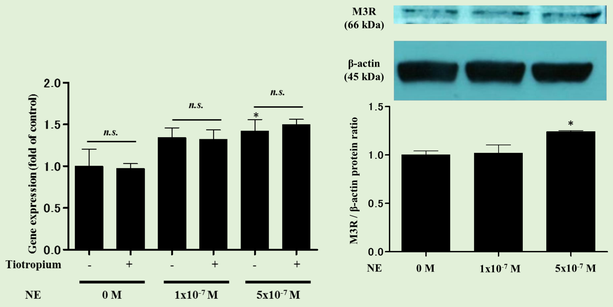


Figure 5. Tiotropium significantly decrease neutrophil elastase (NE) stimulated MUC5AC mRNA expression.



*p<0.05 compared with control; #p<0.05, ##p<0.001 compared with no tiotropium (N=2)

Figure 6. NE significantly increased M3R mRNA expression and protein production.



*p<0.05 compared with control (N=2)

Discussion

Tiotropium non-significantly inhibits IL-13 induced mucin expression, which is consistent with a previous report that showed that tiotropium attenuated goblet cell metaplasia but only with low IL-13 concentration [1].

We confirm that NE-induced MUC5AC expression is significantly attenuated by tiotropium [2].

While IL-13 decreased M3R expression, NE increased M3R expression; this may explain the differential effects of tiotropium on mucin. It is possible that tiotropium will be more effective for the therapy of neutrophil dominant asthma than for "pure" TH2 (IL-13) dominant asthma.

Conclusions

Tiotropium strongly decreased MUC5AC stimulated by NE, but had no effect on IL-13 induced mucin production. This can be explained by differential effects of NE and IL-13 on M3R, the primary muscarinic receptor for tiotropium.

References

1. Kistemaker, LE, et al. Tiotropium attenuates IL-13-induced goblet cell metaplasia of human airway epithelial cells. *Thorax* 2015; 70: 668-76.
2. Arai, N, et al. Inhibition of neutrophil elastase-induced goblet cell metaplasia by tiotropium in mice. *Eur Respir J* 2009; 35: 1164-71.