Effect of theophylline plus beclometasone on lung function in
smokers with asthma—a pilot study

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Word count: 2716

Short title: Exploratory treatment for smoking asthmatics

Key words: asthma, corticosteroid insensitivity, smoking, histone deacetylase, theophylline
**Funding sources:** This project was funded by the Chief Scientists Office (Scotland) & Chest, Heart & Stroke (Scotland). GSK acted as sponsor for the trial.
ABSTRACT

Background
Smoking is common in asthma and is associated with worse asthma control and a reduced therapeutic response to corticosteroids. We hypothesised that treating smokers with asthma with low dose theophylline added to inhaled corticosteroid would enhance steroid sensitivity and thereby improve lung function and symptoms.

Methods
In a double-blind, parallel group exploratory trial 68 asthmatic smokers were randomised to one of three treatments for 4 weeks: inhaled beclometasone (200 mcg per day), theophylline (400 mg per day), or both treatments combined. Outcome measures included change in lung function and asthma control questionnaire (ACQ) scores.

Results
At four weeks, theophylline added to inhaled beclometasone produced an improvement in PEF (39.9 L/min, 95% CI 10.9 to 68.8, p=0.008) and ACQ score (-0.47, -0.91 to -0.04, p=0.033) and a borderline improvement in pre-bronchodilator FEV1 (mean difference 165 ml, -13 to 342, p=0.069) relative to inhaled corticosteroid alone. Theophylline alone improved ACQ score (-0.55, -0.99 to -0.11, p=0.016) but not lung function.

Conclusions
In this pilot study the combination of low dose theophylline and inhaled beclometasone
produced improvements in both lung function and symptoms in a group of smokers with asthma. Larger trials are required to extend and confirm these findings.

(Clinicaltrials.gov number NCT00119496)

Word count: 198
INTRODUCTION

Inhaled corticosteroids are recommended as first-line treatment for chronic persistent asthma [1], although a significant proportion of individuals fail to establish complete control of their asthma despite this approach [2]. Smokers with asthma form part of this poorly controlled group as demonstrated by their increased symptoms [2, 3], increased emergency department visits for asthma [4, 5] and an impaired response to both inhaled and oral corticosteroids [6-9] compared to non-smokers with asthma. The prevalence of smoking in asthma reflects that of the general population and therefore smokers with asthma comprise a large group of patients with poorly controlled disease [10]. Smoking cessation is an effective intervention for smokers with asthma [11], but as sustained quitting rates are low, additional or alternative therapies are needed for individuals with asthma who smoke.

The anti-inflammatory actions of corticosteroids are mediated, in part by recruitment of histone deacetylase-2 (HDAC2), a nuclear enzyme involved in the switching off of activated inflammatory genes [12]. Cigarette smoke reduces HDAC activity in-vitro [13], which could explain corticosteroid insensitivity in smokers with asthma. At standard doses, theophylline produces bronchodilation, whereas low doses increase HDAC activity with associated reductions in inflammatory gene expression [14]. Therefore, we undertook an exploratory clinical trial to examine the effects of low dose theophylline in combination with low dose inhaled corticosteroid and theophylline alone on lung function and other outcomes in comparison to inhaled corticosteroid alone in smokers with asthma.
METHODS

Subjects

Mild to moderate [1] stable asthmatics aged 18 to 60 years on $\leq 1000$ mcg beclometasone (or equivalent) per day who were current smokers ($\geq 5$ cigarettes per day with $\geq 5$ pack years smoking history) were eligible for enrolment. All subjects demonstrated reversible airflow obstruction [15]. Exclusion criteria included diabetes, recent myocardial infarction or other active pulmonary diseases (full criteria available at www.clinicaltrials.gov; NCT00119496). Patients were recruited from general practice, hospital clinics and research databases. The West Glasgow Research Ethics Committee approved the study and all patients gave written informed consent.

Study design

The study was a randomised, prospective, double-blind, double-dummy, active comparator, parallel group design. Subjects taking inhaled corticosteroids alone or in combination with inhaled long acting beta$_2$-agonists completed a step-down in therapy during a six week run in period where they were monitored for loss of asthma control. At the end of a two-week corticosteroid-free period each subject (if deemed to have stable asthma) attended for a randomisation visit, which comprised spirometry, peak expiratory flow (PEF), completion of an asthma control questionnaire (ACQ)[16], induced sputum for differential counts, supernatant and sputum macrophage HDAC
activity and bloods for safety (full blood count, renal and liver function testing) and characterisation (total and specific IgE).

Subjects were then randomised to one of three arms [1] 200mcg/day inhaled hydrofluoroalkane beclometasone dipropionate (Qvar®, IVAX, Runcorn, Cheshire, UK) [Equivalent to approximately 400mcg per day chlorofluorocarbon beclometasone] [17], [2] 400mg/day oral theophylline (Uniphyllin® Continus®, NAPP, Cambridge, UK) or [3] 400mg per/day oral theophylline in combination with 200mcg/day inhaled beclometasone. Pre-bronchodilator lung function was re-assessed at fourteen days and all tests performed at the randomisation visit were repeated after twenty-eight days with the addition of serum theophylline level.

**Measurements**

Lung function assessments conformed to consensus guidelines [15]. Sputum induction, differential count and supernatant analysis were performed as previously [11] with minor modifications to optimise HDAC measurement [18]. Sputum macrophages were encouraged to adhere by plating out in 6 well plates for 1 hour at 37°C and then processed for HDAC activity using a Fluor-de-Lys™ assay kit (Biomol International, Exeter, UK) and analysed using fluorimetry. Sputum supernatants were collected for interleukin (IL)-8, myeloperoxidase (MPO) and Regulated on Activation, Normal T Expressed and Secreted (RANTES)/CCL5 measurement using enzyme immunoassay (IL-8, R&D Systems, Abingdon, UK, MPO, Immundiagnostik, Oxford Biosystems, Oxford, UK, RANTES/CCL5, Invitrogen Ltd, Paisley, UK). Theophylline levels were
assessed using an automated processing system (ARCHITECT c8000, Abbot Diagnostics, Maidenhead, UK). Continuation of smoking was confirmed by the detection of urinary nicotine metabolites at each visit using the SmokeScreen™ system (GFC Diagnostics, Bicester, UK). Subjects were regarded as current smokers if their urine cotinine equivalent concentration was greater than 1.1mg/ml. Treatment compliance was assessed by tablet count, inhaler weight and serum theophylline level.

**Statistical analysis**

The reduced response to inhaled corticosteroids in smokers with asthma meant we were unable to perform standard power calculations. The study was informed by a previous clinical trial employing oral corticosteroids in smokers with asthma [9]. This resulted in our estimation that we needed to recruit 22 subjects per group to detect a 230ml difference in FEV₁ between the treatment arms and to allow for a 10% dropout.

The randomisation schedule was generated in blocks using a validated system (RandAll). The primary endpoint was difference in pre-bronchodilator FEV₁ between the treatments and beclometasone alone at four weeks. Last value obtained was carried forward for analysis. Lung function changes were examined using ANCOVA (incorporating Kenward & Roger’s method for small groups) using SAS v8.2 (SAS Institute Inc, NC, USA). The remaining statistical analysis was performed using Minitab 15 (Minitab Inc. State College, PA, USA). Level of statistical significance was set at <0.05. No adjustment was performed for multiple comparisons. Parametric data was examined using paired t-testing, 2 sided t-testing or ANOVA and non-parametric data
with Mann-Whitney or Kruskal-Wallis testing as appropriate.

RESULTS

A total of 3895 smokers with asthma were invited to participate between August 2005 and May 2007, of whom 294 gave positive responses. Following telephone screening, visits were arranged for 187 subjects and 91 subjects were randomised (Figure 1). Twenty-three subjects were allocated to the inhaled beclometasone alone and theophylline alone groups and twenty-two subjects to the combination of theophylline and inhaled beclometasone. Twenty-three subjects were randomised to another treatment not discussed in this paper. The baseline demographic, clinical (including previous inhaled corticosteroid and long-acting β₂-agonist use) and inflammatory characteristics of each group were well matched (Tables 1 & 2). All the lung function and asthma control questionnaire results presented in the text are changes derived by ANCOVA relative to the inhaled corticosteroid group response.

Lung function

*Theophylline and inhaled beclometasone*

After 4 weeks, the subjects treated with the combination of theophylline and inhaled beclometasone demonstrated a significant improvement in morning PEF (39.9 L/min, 95% CI 10.9 to 68.8, p=0.008) (Figure 2a) and a borderline significant improvement in mean pre-bronchodilator FEV₁ (mean difference 165 ml, 95% CI -13 to 342, p=0.069)
(Figure 2b) and pre-bronchodilator FVC (254 ml, 95% CI 63 to 445, p=0.010). After 2 weeks, low dose theophylline and beclometasone showed a trend for improvement in morning PEF (24.9 L/min, 95% CI –1.5 to 51.2, p=0.064) and pre-bronchodilator FVC (132 ml, 95% CI –23 to 286, p=0.094). There were no detectable differences in other lung function endpoints (Table 3).

**Theophylline**

Theophylline alone did not improve lung function except for post-bronchodilator FVC at 4 weeks (304 ml, 95% CI 5 to 604, p=0.046) (Table 3 & Figure 2 a & b).

**Asthma Control Questionnaire score**

After 4 weeks, the combination of theophylline and inhaled beclometasone produced a significant improvement in ACQ score (-0.47, 95% CI –0.91 to –0.04, p=0.033) (Figure 2c & Table 3). Theophylline alone also reduced the ACQ score (-0.55, 95% CI –0.99 to –0.11, p=0.016) (Figure 2c & Table 3).

**Sputum samples**

*Induced sputum cytology*

Ninety-seven percent of subjects produced a sample adequate for analysis both pre- and post-treatment. Treatment with the combination of theophylline and inhaled
beclometasone was associated with a reduction in the mean absolute (-10.99, 95% CI, -18.15, -1.65, \( p = 0.018 \)) and percentage sputum lymphocyte count (Table 3). No other relative treatment differences were observed.

Inflammatory biomarkers in sputum

At 4 weeks, treatment with theophylline alone was associated with a reduction in sputum supernatant IL-8 (-1201.3 pg/ml, 95% CI, -2409.6, -276.6, \( p = 0.009 \)) and MPO (-215.0 ng/ml, 95% CI, -556.0, -36.7, \( p = 0.026 \)) (Table 3). No difference was detected in RANTES/CCL5 levels following treatment with theophylline and inhaled corticosteroid (-0.131 pg/ml, 95% CI, -0.849, 0.528). No other significant differences were detectable.

HDAC activity

HDAC activity was measurable for a subgroup within each treatment group [inhaled beclometasone n=4, theophylline alone n=7, theophylline and inhaled beclometasone n=7]. Nearly all samples obtained had a low level of HDAC activity. No difference was observed between the groups at baseline or after treatment (Tables 1 & 2 and Figure 3).

Serum theophylline levels

The mean serum concentration for the theophylline alone group (4.9 \( \mu g/ml \), SD 2.4) was similar to that achieved in the theophylline and inhaled beclometasone group (4.3
μg/ml, SD 2.0).

**Compliance**

Ninety percent of the subjects who completed the study achieved ≥80% compliance with therapy.

**Adverse events**

Two serious adverse events occurred during the trial. Both occurred in the theophylline alone arm. One subject was admitted to hospital with viral meningitis, a condition felt to be unrelated to the medication and another with chest pain due to gastro-oesophageal reflux (a pre-existing condition), which was considered as exacerbated by the treatment. Neither subject withdrew from the study. There were two withdrawals due to adverse events. One each occurred in the inhaled beclometasone alone (diarrhoea and vomiting) and theophylline and inhaled beclometasone (headache) arms. The frequency of headache was equal between the groups (6 for theophylline and inhaled corticosteroids, 7 for theophylline and 5 for low dose beclometasone). Gastrointestinal upset was common in the theophylline alone group with 14 episodes being reported. Two subjects reported nausea whilst on theophylline and low dose beclometasone. Pharyngitis was reported by 3 subjects in the low dose beclometasone alone group.
DISCUSSION

The therapeutic response to inhaled corticosteroids is impaired in smokers with asthma [6, 7, 8], highlighting the need for alternative treatment approaches for this large subgroup of asthma. Based on previous research [19, 20] we hypothesised that the addition of low dose theophylline to inhaled corticosteroid would improve lung function to a greater degree than that produced with inhaled corticosteroid alone. This study found that the combination of theophylline and inhaled corticosteroid produces improvements in indices of lung function and asthma control score in smokers with asthma.

After four weeks treatment with theophylline in combination with inhaled beclometasone was associated with a large improvement in both pre-bronchodilator PEF and FVC and a borderline significant increase in pre-bronchodilator FEV$_1$. A post hoc power calculation, based on the between patient standard deviation (46.4 l/min) for the theophylline and inhaled corticosteroid group, reveals that 23 subjects per group would be required to provide 80% power for the detection of a 40 litre/min difference in PEF between the combination and inhaled corticosteroid alone groups. The improvement seen in both PEF and FVC (and the associated drop in ACQ score) suggest that the increase in FEV$_1$ in the group treated with low dose theophylline and inhaled beclometasone is likely to be a real effect. The size of the improvement seen following treatment with low dose theophylline and inhaled beclometasone is also likely to be clinically significant as it is larger [21] or equivalent [22] to the improvement in PEF seen when long acting $\beta_2$-agonists are added to inhaled corticosteroids in non-
smoking asthmatics. Furthermore, the improvement in PEF demonstrated here is much larger than that seen with montelukast [8] and high dose inhaled corticosteroids [7] in previous studies in smokers with asthma. The improvements in lung function with low dose theophylline and inhaled beclometasone were also associated with a reduction in ACQ score, just below the clinically significant threshold of 0.5 [23]. Given these findings further research should be carried out using low dose theophylline and inhaled corticosteroid to confirm and extend our understanding of the efficacy of this combination in smokers with asthma.

Theophylline alone did not produce any significant changes in pre-bronchodilator lung function. Nevertheless low dose theophylline treatment did increase post-bronchodilator FVC, produce a clinically significant reduction in ACQ score and a reduction in sputum supernatant cytokines. Previous research has demonstrated that theophylline has a similar effect in subjects with COPD [24]. Theophylline has many modes of action including non-specific phosphodiesterase inhibition and adenosine receptor antagonism [25] and both of these mechanisms could produce bronchodilation. However we believe that the improvement in lung function with low dose oral theophylline in combination with inhaled beclometasone is unlikely to be due to a bronchodilating effect of theophylline alone, given the absence of a statistically significant improvement in pre bronchodilator lung function with low dose theophylline alone. A previous study has demonstrated superiority for the combination of low dose theophylline and inhaled beclometasone to high dose beclometasone in non smokers with moderate persistent asthma [26]. Our results confirm this finding and we conclude that there appears to be a synergistic interaction between low dose theophylline and
corticosteroid in smokers with asthma.

Given the many suggested mechanisms of action of theophylline there are several ways by which low dose theophylline could synergise with corticosteroids to improve lung function in smokers with asthma. One potential mechanism we attempt to address in this study is the ability of low dose theophylline to restore HDAC activity. *In-vitro* HDAC activity can be reduced by cigarette smoke and restored by low doses of theophylline [13, 14]. Therefore we hypothesised that reduced HDAC activity was responsible for the reduced corticosteroid response seen in smokers with asthma and that this could be restored by low dose theophylline resulting in an improved steroid response. The serum concentration of theophylline achieved in the subjects was within the range previously demonstrated to stimulate HDAC activity. We were not able to confirm an increase in HDAC activity in those subjects treated with theophylline and inhaled corticosteroids despite the subjects being able to produce specimens of sufficient quality for differential counting and supernatant analysis. The reason for this failure was that the number of sputum macrophages harvested for HDAC was low and hence at the detection limit of the technique. Future work examining theophylline in smokers with asthma to address the underlying mechanism/s responsible may need to use bronchoalveolar lavage samples to ensure sufficient macrophages for HDAC analysis. As theophylline can also act as a non-specific phosphodiesterase inhibitor and an adenosine receptor antagonist, the contribution of these (and other) mechanisms to synergism between theophylline and inhaled beclometasone needs to be examined in smokers with asthma.
In the present study, we have also demonstrated that treatment with theophylline and inhaled beclometasone is associated with a reduction in sputum lymphocytes. How this contributes to an improved response to inhaled corticosteroids is unclear. A possible explanation is that the reduction we have observed is merely due to the group treated with theophylline and inhaled corticosteroid having a slightly higher sputum lymphocyte count at baseline (albeit non-significantly different). Previous work addressing the reproducibility of induced sputum counts has also demonstrated that sputum lymphocyte counts display lower reproducibility compared to eosinophils and neutrophils [27] so we may be observing the inherent variability of this aspect of induced sputum. However low dose theophylline has been demonstrated to reduce airway lymphocyte numbers associated with a reduction in the late asthmatic response to allergen challenge and an alteration in CD4/CD8 T lymphocyte ratio [28]. Therefore the reduction in sputum lymphocytes we have observed may be a true effect.

Finally, it would be noted that apart from the main trial endpoint, there were 40 other comparisons made to produce the data presented in table 3. Although it is usual practice to allow for this in the analysis, by performing adjustments of the alpha value or using 99% confidence intervals only, we presented unadjusted p values in this paper as the study is a pilot [see online depository file for results of analysis adjusted for multiple comparisons]. The aim has been to identify associations that would need to be examined in future studies.

In conclusion this pilot study demonstrates improvements in both lung function and asthma control from the addition of low dose theophylline to inhaled corticosteroids in a
group of smokers with mild to moderate asthma. Important questions that need to be addressed in future include the relative performance of the combination of low dose theophylline and low dose inhaled corticosteroid to high dose inhaled corticosteroid, the effect of lower doses of theophylline and hence the lowest effective dose and the relative performance of the combination of low dose theophylline and inhaled corticosteroid to the combination of long acting beta agonist and inhaled corticosteroids. Larger trials examining these important management issues should be performed to allow confirmation and extension of our findings.

ACKNOWLEDGMENTS

This study is dedicated to the memory of Dr Stuart Wood who died suddenly shortly after the commencement of recruitment. An employee of GSK blinded to the allocation of the individual patients performed the lung function statistical analysis. We would like to express our gratitude to Mr Brian Rae, Greater Glasgow and Clyde Primary Care R&D, for his help & support with this project. We would also like to thank the GPs from Greater Glasgow and North and South Lanarkshire and the volunteers who participated in the study.
REFERENCES


17. Vanden Burgt J, Busse, WW, Martin, RJ, Szefler, SJ, Donnell, D. Efficacy and safety overview of a new inhaled corticosteroid, QVAR (hydrofluoroalkane-
beclomethasone extrafine inhalation aerosol), in asthma. *Journal of Allergy and Clinical Immunology* 2000: 106(6): 1209-1226.


Table 1: Baseline demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inhaled beclometasone</th>
<th>Theophylline &amp; Inhaled beclometasone</th>
<th>Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>23</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42 (36, 53)</td>
<td>44 (31, 52)</td>
<td>46 (38, 50)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female (% of total)</td>
<td>61</td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>BMI (kg/m²) Mean (range)</td>
<td>25.5 (18.4, 34.2)</td>
<td>26.0 (17.3, 36.1)</td>
<td>26.6 (18.6, 37.1)</td>
</tr>
<tr>
<td>Pack years</td>
<td>24 (15, 30)</td>
<td>25 (11, 40)</td>
<td>30 (15, 35)</td>
</tr>
<tr>
<td>Duration of asthma (years)</td>
<td>16 (8, 31)</td>
<td>15 (9, 21)</td>
<td>16 (9, 30)</td>
</tr>
<tr>
<td>Inhaled corticosteroid use at screening (% of total)</td>
<td>65</td>
<td>68</td>
<td>74</td>
</tr>
<tr>
<td>Dose, beclometasone equivalent (mcg)</td>
<td>800 (400, 800)</td>
<td>800 (400, 950)</td>
<td>400 (400, 900)</td>
</tr>
<tr>
<td>LABA use at screening (%)</td>
<td>26</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>Specific IgE antibody positive (%)</td>
<td>61</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Total IgE level (IU/ml)</td>
<td>87 (34, 396)</td>
<td>91 (31, 383)</td>
<td>40 (9, 346)</td>
</tr>
<tr>
<td>Spirometry (Pre-BD) FEV₁ (% predicted)</td>
<td>75 (72, 89)</td>
<td>78 (65, 84)</td>
<td>73 (64, 84)</td>
</tr>
<tr>
<td>Reversibility FEV₁ % improvement</td>
<td>16 (13, 20)</td>
<td>15 (14, 18)</td>
<td>18 (14, 24)</td>
</tr>
<tr>
<td>Asthma Control Questionnaire score (0 to 6) Mean (SD)</td>
<td>1.8 (0.9)</td>
<td>1.8 (0.7)</td>
<td>2.1 (0.5)</td>
</tr>
</tbody>
</table>

Data presented as median (interquartile range) unless stated otherwise.

Abbreviations; SD; standard deviation, BMI; Body Mass Index, PEF; Peak expiratory flow rate, FEV₁; Forced expiratory volume in 1 second, pre BD; pre bronchodilator, ACQ; Asthma Control Questionnaire score (range, 0 to 6, with higher scores indicating worse asthma control), IgE; immunoglobulin E, 95% CI; 95% confidence intervals, ppb; parts per billion, LABA; long-acting β₂-agonist.
Table 2-Baseline sputum counts and HDAC activity

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Inhaled beclometasone</th>
<th>Theophylline &amp; Inhaled beclometasone</th>
<th>Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sputum total cell count</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10⁶)</td>
<td>4.3 (2.6, 7.3)</td>
<td>5.1 (3.1, 9.4)</td>
<td>6.0 (2.4, 16.1)</td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>0.9 (0.3-1.6)</td>
<td>0.8 (0.4-1.8)</td>
<td>1.3 (0.5-2.3)</td>
</tr>
<tr>
<td>absolute count (10⁴ cells)</td>
<td>2.1 (0.8, 5.8)</td>
<td>3.7 (1.9, 19.5)</td>
<td>6.3 (1.7, 28.4)</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>25.5 (9.6-44.6)</td>
<td>23.5 (8.6-42.3)</td>
<td>16.6 (8.4-40.3)</td>
</tr>
<tr>
<td>absolute count (10⁴ cells)</td>
<td>122.7 (25, 188)</td>
<td>83.0 (35, 302)</td>
<td>80.0 (25, 323)</td>
</tr>
<tr>
<td><strong>Macrophages</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>52.8 (32.0-64.4)</td>
<td>45.1 (38.1-60.8)</td>
<td>52.1 (39.1-64.3)</td>
</tr>
<tr>
<td>absolute count (10⁴ cells)</td>
<td>184.2 (96, 437)</td>
<td>271.8 (165, 452)</td>
<td>78.0 (147, 729)</td>
</tr>
<tr>
<td><strong>Lymphocytes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>1.3 (0.6, 2.6)</td>
<td>1.6 (1.0, 2.7)</td>
<td>1.0 (0.5, 2.5)</td>
</tr>
<tr>
<td>absolute count (10⁴ cells)</td>
<td>4.9 (2.3, 11.2)</td>
<td>12.4 (3.2, 20.7)</td>
<td>7.1 (1.5, 22.6)</td>
</tr>
<tr>
<td><strong>Bronchial epithelial cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>10.5 (8.3, 15.4)</td>
<td>16.4 (8.0, 28.9)</td>
<td>12.3 (6.1, 27.2)</td>
</tr>
<tr>
<td>absolute count (10⁴ cells)</td>
<td>40.7 (20.5, 99.4)</td>
<td>119.1 (63.8, 157.6)</td>
<td>83.7 (22.1, 168.2)</td>
</tr>
<tr>
<td><strong>HDAC activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFU/10⁶ cells mean (95% CI)</td>
<td>2.25 (0.54, 3.95)</td>
<td>3.75 (0.34, 7.16)</td>
<td>3.62 (0.61, 6.64)</td>
</tr>
</tbody>
</table>

Data presented as median (interquartile range) unless stated otherwise.

Abbreviations; SD; standard deviation, 95% CI; 95% confidence intervals, AFU; arbitrary fluorescence units. HDAC samples per group, inhaled beclometasone n=10, theophylline n=14, theophylline & inhaled beclometasone n=12.
<table>
<thead>
<tr>
<th>Change (relative to inhaled beclometasone)</th>
<th>Theophylline &amp; inhaled beclometasone</th>
<th>Theophylline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Pre BD FEV₁ ml (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>133 (-27, 293)</td>
<td>52 (-109, 214)</td>
</tr>
<tr>
<td>Day 28</td>
<td>165 (-13, 342)</td>
<td>128 (-51, 307)</td>
</tr>
<tr>
<td>Δ Pre BD PEF L/min (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>25 (-1, 51)</td>
<td>6 (-20, 33)</td>
</tr>
<tr>
<td>Day 28</td>
<td>40 * (11, 69)</td>
<td>22 (-7, 51)</td>
</tr>
<tr>
<td>Δ Pre BD FVC ml (95% CI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>132 (-23, 286)</td>
<td>15 (-141, 171)</td>
</tr>
<tr>
<td>Day 28</td>
<td>254 * (63, 445)</td>
<td>176 (-16, 368)</td>
</tr>
<tr>
<td>Δ ACQ score (95% CI)</td>
<td>-0.47 * (-0.91, -0.04)</td>
<td>-0.55 * (-0.99, -0.11)</td>
</tr>
<tr>
<td>Δ Sputum total cell count Cells x 10⁶ (95% CI)</td>
<td>-2.0 (-6.3, 1.7)</td>
<td>-1.7 (-6.2, 2.1)</td>
</tr>
<tr>
<td>Δ Sputum eosinophil % (95% CI)</td>
<td>0.0 (-1.1, 0.6)</td>
<td>-0.6 (-1.7, 0.3)</td>
</tr>
<tr>
<td>Absolute (10⁴) (95% CI)</td>
<td>-1.62 (-9.58, 1.82)</td>
<td>-5.53 (-17.87, 1.68)</td>
</tr>
<tr>
<td>Δ Sputum neutrophil % (95% CI)</td>
<td>0.3 (-12.3, 17.7)</td>
<td>-2.5 (-22.5, 12.8)</td>
</tr>
<tr>
<td>Absolute (10⁴) (95% CI)</td>
<td>46.8 (-65.1, 236.2)</td>
<td>-16.0 (-199.5, 116.1)</td>
</tr>
<tr>
<td>Δ Sputum macrophage % (95% CI)</td>
<td>0.5 (-11.8, 11.3)</td>
<td>-2.5 (-21.0, 15.3)</td>
</tr>
<tr>
<td>Absolute (10⁴) (95% CI)</td>
<td>-52.7 (-251.4, 118.7)</td>
<td>-0.9 (-250.0, 186.3)</td>
</tr>
<tr>
<td>Δ Sputum lymphocyte % (95% CI)</td>
<td>-0.8 * (-1.4, -0.1)</td>
<td>-0.6 (-1.3, 0.2)</td>
</tr>
<tr>
<td>Absolute (10⁴) (95% CI)</td>
<td>-10.99 * (-18.15, -1.65)</td>
<td>-3.98 (-10.30, 1.36)</td>
</tr>
<tr>
<td>Δ Sputum bronchial epithelial cell % (95% CI)</td>
<td>1.2 (-5.8, 7.4)</td>
<td>-1.0 (-11.3, 5.7)</td>
</tr>
<tr>
<td>Absolute (10⁴) (95% CI)</td>
<td>-20.9 (-85.1, 50.5)</td>
<td>-12.9 (-100.8, 57.3)</td>
</tr>
<tr>
<td>Δ Sputum IL-8 pg/ml (95% CI)</td>
<td>-562.5 (-2131.0, 131.4)</td>
<td>-1201.3 * (-2409.6, -76.6)</td>
</tr>
<tr>
<td>Δ Sputum MPO ng/ml (95% CI)</td>
<td>-126.6 (-433.9, 58.1)</td>
<td>-215.0 * (-556.0, -36.7)</td>
</tr>
</tbody>
</table>
Δ HDAC activity | AFU/10^6 cells (95% CI) | -3.5 (-23.7, 5.0) | -2.2 (-23.4, 3.5)

*; p<0.05. Δ = change. Lung function data and ACQ score differences are difference of adjusted means with adjustment for baseline measurement (ANCOVA). HDAC samples per group; inhaled beclometasone n=4, theophylline alone n=7, theophylline and inhaled beclometasone n=7. Changes in ACQ score, sputum cell counts, supernatant mediator levels and HDAC activity refer to change from randomisation visit to day 28.
Figure 1 - CONSORT diagram

74 practices
3896 invitation letters
294 responses

Screened (n=187)

Excluded (n=96):
Not meeting inclusion criteria (n=95)
Pregnancy (n=1)
Randomised to another treatment (n=23)

Randomised (n=68)

Inhaled beclometasone
Allocated n=23
Received n=23
Did not receive n=0

Lost to follow up (n=0)
Discontinued intervention (n=2)
Analysed (n=21)
Excluded from analysis (n=0)

Theophylline & beclometasone
Allocated n=22
Received n=22
Did not receive n=0

Lost to follow up (n=0)
Discontinued intervention (n=2)
Analysed (n=20)
Excluded from analysis (n=0)

Theophylline
Allocated n=23
Received n=23
Did not receive n=0

Lost to follow up (n=1)
Discontinued intervention (n=2)
Analysed (n=20)
Excluded from analysis (n=0)

SAE
Theophylline + inhaled beclometasone n=0
Theophylline n=2 (viral meningitis & chest pain secondary to reflux oesophagitis)
Inhaled beclometasone n=0
Figure 2

2a - Change in PEF by 28 days of treatment

2b - Change in FEV1 by 28 days of treatment
2c - Change in Asthma Control Questionnaire (ACQ) score by 28 days of treatment

Changes presented are individual plots of change from randomisation to 28 days of treatment (paired t-test | error bars represent 95% confidence intervals). p-values were derived from comparison of groups to inhaled beclometasone change using ANCOVA.

Abbreviations: ICS, inhaled corticosteroid (beclometasone), Theo; theophylline, Theo + ICS; theophylline and inhaled corticosteroid (beclometasone) combination, BD; Bronchodilator
Figure 3- Change in sputum Histone De-Acetylase (HDAC) activity by 28 days of treatment

Figure represents change in HDAC activity from randomisation to 28 days of treatment. Individual plots of HDAC activity shown. Error bars represent 95% confidence intervals. Figure key: ICS, inhaled beclomethasone, Theo, theophylline, Theo + ICS, theophylline and inhaled beclomethasone combination.