The PDE4 inhibitor roflumilast reduces sputum neutrophil and eosinophil numbers in patients with COPD

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See also page 17.
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Abstract

Roflumilast is a targeted, oral, once-daily administered phosphodiesterase 4 (PDE4) inhibitor with clinical efficacy in COPD. Results from in vitro studies with roflumilast indicate that roflumilast has anti-inflammatory properties that may be applicable for the treatment of COPD. In this cross-over study, 38 patients with COPD (mean (SD) age 63.1 (7.0) y, post-bronchodilator FEV₁ 61.0 (12.6) %predicted) received 500 µg roflumilast or placebo once daily for 4 weeks. Induced sputum samples were collected prior to and after 2 and 4 weeks of treatment. Differential and absolute cell counts were determined in whole sputum samples. Markers of inflammation were determined in sputum supernatants and blood. Spirometry was performed weekly. Roflumilast significantly reduced the absolute number of neutrophils and eosinophils per gram of sputum, compared with placebo, by 35.5% (95%CI 15.6, 50.7; p=0.0017) and 50.0% (26.8, 65.8; p=0.0005), respectively. The relative proportion of sputum neutrophils and eosinophils was not affected by treatment (p>0.05). Soluble IL-8, neutrophil elastase, ECP and α2-macroglobulin in sputum, together with TNFα release from blood cells, were significantly reduced by roflumilast compared to placebo treatment (p<0.05 for all). Post-bronchodilator FEV₁ improved significantly during roflumilast as compared to placebo treatment. The mean difference between treatments was 68.7 (12.9, 124.5) mL (p=0.018). In conclusion, PDE4 inhibition by roflumilast treatment for 4 weeks reduced the number of neutrophils and eosinophils, as well as soluble markers of neutrophilic and eosinophilic inflammatory activity in induced sputum samples of COPD patients. This anti-inflammatory effect may in part explain the concomitant improvement in post-bronchodilator FEV₁.

[249 words]
Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation, which is not fully reversible (1) and is associated with an abnormal inflammatory response in the airways (2). The inflammatory infiltrate within the airway lumen in COPD consists mainly of neutrophils (3). Airway neutrophilia is reflected by sputum neutrophilia, and also indicative of systemic inflammation (4;5). Furthermore, in COPD patients, elevated levels of pro-inflammatory cytokines related to neutrophil activity are evident in sputum and the systemic circulation, including interleukin (IL) 8 (6) (associated with enhanced neutrophil chemotaxis), neutrophil elastase (7) (involved in connective tissue degradation), tumour necrosis factor (TNF) α (8) (involved in inflammatory cell activation), and circulating adhesion molecule E-selectin (9) (important in rolling of leukocytes to the endothelium).

At present, treatment options for COPD are limited and consist of bronchodilators alone or in combination with inhaled corticosteroids (1). Treatment with inhaled steroids only slightly ameliorates the accelerated decline in FEV$_1$ in these COPD patients (10) but, in combination with long-acting bronchodilators, reduces inflammatory cell numbers in the bronchial wall and sputum (11).

A novel approach for therapeutic intervention in COPD is through inhibition of phosphodiesterase 4 (PDE4) activity. PDE4 is an intracellular enzyme involved in the degradation of the second messenger cyclic adenosine monophosphate (cAMP) (12;13). Elevated levels of this second messenger relax airway smooth muscle and modulate inflammatory cell activity (14). PDE4 is expressed in inflammatory cells and airway smooth muscle, while basophils, mast cells, monocytes/macrophages, T lymphocytes and smooth muscle cells also express PDE3 (14). Roflumilast is a targeted PDE4 inhibitor, which is under investigation for treatment of COPD and bronchial asthma. Roflumilast ameliorates the activity of various inflammatory cells in
vitro (15), and reduces pulmonary inflammation in complex in vivo animal models (16;17). Furthermore, roflumilast has clinical effects in patients with COPD. Treatment for 24 weeks improves lung function and reduces the number of mild exacerbations (18). However, at present it is unknown whether roflumilast has anti-inflammatory properties in patients with COPD.

We hypothesized that roflumilast has anti-inflammatory effects in COPD patients. Therefore, the aim of this study was to examine the efficacy of oral roflumilast treatment (500 µg once daily for four weeks) on the reduction the percentage of sputum neutrophils in patients with COPD as compared to placebo. Secondary endpoints, including the effect of roflumilast versus placebo on FEV₁, absolute numbers of neutrophils in sputum, other inflammatory cell numbers and percentages in sputum, and markers of activation of inflammatory cells (IL-8, neutrophil elastase, lactoferrin and eosinophil cationic protein [ECP]) and markers of microvascular leakage (α2-macroglobulin) in sputum supernatant were also examined. Finally, the effect of oral roflumilast on systemic markers of inflammation was studied by measurement of TNFα production by whole blood cultures following LPS stimulation and soluble E-selectin levels in serum.
Methods

Patients

Patients with a history of COPD (1) for at least one year were invited to participate and had to fulfill the following criteria: age 45-75 y, current smokers or ex-smokers (stable for ≥6 months) with a smoking history ≥10 packyears, pre-bronchodilator FEV₁/FVC ≤70%, post-bronchodilator FEV₁ 35-75 %predicted, reversibility in FEV₁ <12% or <200 mL from pre-bronchodilator value, sputum neutrophilia (>45% of non-squamous cells) and no exacerbation or upper respiratory tract infection 4 weeks prior to inclusion. Short-acting bronchodilators were allowed during the study whereas long-acting bronchodilators, theophylline (2 weeks), inhaled and/or oral corticosteroids (4 weeks) were discontinued prior to inclusion. The study was approved by the Medical Ethics Committee of the Leiden University Medical Center and performed according to the Declaration of Helsinki (2). All patients gave written informed consent.

Design

This study had a randomized, double-blind, placebo-controlled, cross-over design, consisting of two treatment periods of 4 weeks with a wash-out period of 4-6 weeks between treatments (figure 1). Following inclusion, patients entered a run-in period of 2 weeks during which placebo tablets were used. Patients compliant to the study medication (>70% of tablets used), and with lung function and sputum within the criteria of inclusion, were randomized to receive either roflumilast 500 µg or placebo once daily for four weeks by means of a concealed, computer generated randomization list.

During each treatment period, patients visited the laboratory at weekly intervals for measurement of lung function, compliance check, and sampling of blood for determination of E-selectin and whole blood stimulation assay with
lipopolysaccharide for measuring TNFα. Blood samples were drawn at wk 0 and wk 8 prior to administration of medication (total volume 49 mL), whereas at all other visits this was approximately 1 h after the intake of study medication (total volume 34.5 mL). Sputum samples were collected at inclusion, start of each treatment period, and at 2 and 4 weeks of treatment. All patients were supplied with salbutamol MDI 100 µg/puff for on demand symptom relief. Patients withheld salbutamol, smoking and caffeine containing beverages for 6 hours, and anti-cholinergics for 8 hours prior to lung function measurements. ECG, vital signs, and clinical laboratory parameters were performed at inclusion, beginning and end of each treatment period. Adverse events were monitored throughout the study.

Methods

FEV₁ was recorded from maximal expiratory flow-volume curves on a calibrated pneumotachograph according to standards (3;4). Reversibility in FEV₁ was determined 30 minutes after inhalation of 400 µg salbutamol.

Sputum induction was performed by inhalation of 4.5% hypertonic saline aerosols (5). Whole sputum samples were processed (6). Differential cell counts were expressed as percentage of non-squamous cells. Absolute cell numbers were calculated as (%cell x total cell count)/sputum weight. IL-8, lactoferrin, α₂-macroglobulin and neutrophil elastase were measured by enzyme linked immunosorbent assays (ELISA); ECP by fluoroimmunoassay.

E-selectin concentrations in serum were measured by ELISA. TNFα release was assessed in whole blood cultures of heparinized blood that were stimulated with lipopolysaccharide (LPS) essentially as described (7).
Safety

This trial involved extensive safety and tolerability assessments, including vital signs, clinical lab and adverse event monitoring.

Statistical analysis

Demographic data are presented as mean (standard deviation) or median (minimum, maximum). Non-normally distributed data were log-transformed prior to analysis.

Primary endpoint of the study was the reduction in percentage of sputum neutrophils. Further analyses included FEV₁, absolute number of sputum neutrophils, other inflammatory cell numbers and percentages in sputum, markers of activation of inflammatory cells (IL-8, neutrophil elastase, lactoferrin and ECP), markers of microvascular leakage (α2-macroglobulin) in sputum supernatant, and systemic markers of inflammation including TNFα production by whole blood cultures following LPS stimulation and soluble E-selectin levels in serum. The sample size was calculated using a two-sided α of 5% and power of 80%, together with a conservative estimation of correlation between paired observations (0.5). It was calculated that with a sample size of 32 (evaluable) patients, we would be able to detect a treatment effect with a standard deviation of 1.9 times the mean difference. Longitudinal data were analysed by repeated measures analysis, with correction for period effects (ANCOVA). Effect estimates between treatments were calculated from linear mixed models and presented as point estimate with 95% confidence intervals. Confidence intervals of point estimates not including 1 for log-transformed data and not including 0 for normally distributed data were interpreted as statistically significant. A p-value <0.05 was considered as the level of statistical significance for the latter analyses.
Results

Participant flow

Forty-four patients with COPD were included in the study between January 2001 and March 2002. Of these, 41 patients were randomized. In two patients, FEV\textsubscript{1} reversibility criteria were not met, whereas in one patient the neutrophilia criteria were not met. These patients were excluded from the analyses. Therefore, data from a total of 38 patients were used for the efficacy analysis. During treatment, six patients dropped out of the study due to withdrawal of consent (n=1), exacerbation of COPD requiring additional treatment (n=2), and adverse events to the study medication (n=3, see below). Baseline characteristics of the patients who contributed to the efficacy analysis are shown in table 1 and 2.

Lung function

Pre- and post-bronchodilator FEV\textsubscript{1} improved significantly during roflumilast treatment (figure 2). The change in both pre- and post-bronchodilator FEV\textsubscript{1} was significantly different between roflumilast and placebo treatment (figure 2, p<0.0001 and p=0.018, respectively). The point estimate of difference between the treatments (95% confidence interval) was 79.5 mL (54.0, 105.1 mL) for pre-bronchodilator FEV\textsubscript{1} and 68.7 mL (12.9, 124.5 mL) for post-bronchodilator FEV\textsubscript{1}.

Inflammatory cells in sputum

At baseline, total inflammatory cell count comprised primarily of neutrophils (74%). The inflammatory cell load in the airways, as reflected by the total non-squamous cell count in sputum samples, decreased during roflumilast treatment whereas it increased during placebo (difference between treatments p=0.0023, figure 3). Overall, total cell count decreased significantly by 34% during roflumilast treatment as compared to placebo (table 3). In particular, roflumilast treatment was associated with a significant decrease in neutrophil numbers in sputum (p=0.0017, figure 3, table...
In addition, roflumilast treatment reduced sputum eosinophil (p=0.0005, figure 3, table 3), macrophage (p=0.067, table 3) and lymphocyte numbers (p=0.022, table 3). Sputum weight was not affected by roflumilast or placebo treatment (p=0.68, table 3).

In contrast to absolute cell numbers, differential cell counts for neutrophils, macrophages and lymphocytes, expressed as percentage of total non-squamous cells were not affected by the treatments (table 3). Percentage eosinophils tended to be reduced by roflumilast treatment compared to placebo (p=0.052, table 3).

**Markers of inflammation and microvascular leakage in sputum supernatant**

The levels of the neutrophil chemoattractant IL-8 and of the neutrophil degranulation product (neutrophil elastase) decreased significantly during roflumilast treatment as compared to placebo (p<0.05, figure 4, table 3). In contrast to neutrophil elastase, levels of lactoferrin (a marker of neutrophil activity but also produced by submucosal glands), were not affected during treatment (p=0.18, figure 4, table 3). Eosinophil activity, as reflected by ECP levels in sputum, was significantly reduced by roflumilast treatment versus placebo (p=0.015, figure 4, table 3). Roflumilast significantly reduced microvascular leakage, as measured by α2-macroglobulin, compared to placebo (p=0.0003, figure 4, table 3).

**Markers of inflammation in the circulation**

TNFα secretion by whole blood cultures following *ex vivo* stimulation by LPS was significantly reduced during roflumilast treatment as compared to placebo (p=0.047, table 3). In contrast, E-selectin levels in the circulation were not different between the two treatments (p=0.23, table 3).
There was no carry-over effect from the first to the second treatment period in any statistical analysis presented.

**Adverse events**

Thirty-three patients (87%) reported at least one adverse event during roflumilast treatment, while 26 patients (67%) reported such events during placebo treatment (table 4). These adverse events were of mild or moderate intensity and transient. No serious adverse events were reported. Most frequent adverse events under roflumilast were diarrhoea and headache, which are part of the known side effect profile of roflumilast. Three patients discontinued the study due to adverse events, which were all during roflumilast treatment. Their adverse events resolved upon withdrawal of the study medication. There were no relevant findings regarding laboratory, ECG and vital signs.
Discussion

This cross-over, placebo-controlled study is the first to examine anti-inflammatory and pulmonary effects of the oral PDE4 inhibitor roflumilast in patients with COPD. Our results show that roflumilast treatment reduces the inflammatory activity in induced sputum, a surrogate for airway inflammation. The absolute number of sputum inflammatory cells, including neutrophils, eosinophils and lymphocytes, were reduced by 35 to 50% during the four week treatment with roflumilast versus placebo, with such a tendency for number of macrophages. As a result, the total cell count was significantly decreased by 34%. Furthermore, this was accompanied by a reduction in the levels of chemoattractant stimuli for neutrophils, cellular activity of eosinophils and neutrophils in sputum, as well as microvascular leakage.

Concomitant with these changes in sputum inflammation were a decrease TNFα release in blood, and an improvement in pulmonary function, as assessed by FEV₁. We conclude that roflumilast has anti-inflammatory effects in COPD patients, which is accompanied by an improvement in lung function.

Our data extend the findings of Gamble et al. who showed that treatment with PDE4 inhibitor cilomilast for 12 weeks significantly reduces inflammatory cell numbers in bronchial biopsy specimens from COPD patients (26). In the present study as well as in the study of Gamble et al., treatment with a PDE4 inhibitor did not change differential cell counts in sputum samples. This may imply that absolute cell numbers rather than relative cell counts should be assessed to monitor anti-inflammatory effects of treatment in patients with COPD, as has been suggested previously (27). Furthermore, we have shown that roflumilast significantly attenuates cellular activity within the airways of COPD patients, as reflected by reductions in the level of IL-8, neutrophil elastase and ECP, and reduces microvascular leakage. Such anti-inflammatory effects in COPD patients have previously been shown for the PDE4 inhibitor BAY 19-8004, although treatment with this compound was not associated
with an effect on cellular inflammation (28). Lactoferrin levels were not significantly changed by roflumilast. However, the effect size was in the same order as for the other inflammatory parameters studied, which may indicate that signal to noise ratio was too large for lactoferrin, in our study with a relatively small sample size.

In addition to anti-inflammatory activity, we have demonstrated that roflumilast improves both pre- and post-bronchodilator FEV₁ in the same COPD patients. This finding confirms recently reported data that roflumilast provides improvement in FEV₁ of about 100 mL together with a 34% reduction in the number of exacerbations in COPD patients with lung function that is poorly reversible to bronchodilators (18). This improvement in FEV₁ may in part be explained by the parallel reduction in number and activation status of neutrophils and eosinophils in induced sputum samples of these patients with COPD.

A large proportion of patients experienced adverse events during roflumilast and placebo treatment, which is in line with previous studies (18). Most frequently, adverse events during roflumilast were diarrhoea and headache, which are typically PDE4-related and part of the known side effect profile of roflumilast. All adverse events were transient and none of the patients experienced a serious adverse event. Although three patients discontinued the study due to adverse events during roflumilast treatment, their adverse events resolved upon withdrawal of the study medication. Whether roflumilast could be tolerated by these patients following gradual increase of the dose, was not investigated in the present study.

The present study has potential limitations. First, patients with sputum neutrophils $\geq 45\%$ were included in the study to ensure that there would be room for improvement in sputum neutrophils during treatment. Ninety five percent of (ex)smokers with COPD had sputum neutrophilia of this magnitude and only two patients were
excluded from participation due to a lower percentage of neutrophils. Therefore, it is likely that our results can be generalized to the majority of COPD patients. Second, it has been suggested recently that a window of at least six months should be considered to evaluate the effect of therapy in COPD (29). Remarkably, even though our patients were treated for only four weeks, the present results show a clear treatment effect within this short time, as has been demonstrated previously for roflumilast (18). The treatment effect was corrected for variation during placebo treatment. During placebo treatment, a relative worsening of inflammation was observed as opposed to a relative attenuation during roflumilast treatment, resulting in a mean overall difference between roflumilast and placebo, which was significant for various cellular and soluble markers of inflammation. Furthermore, the statistical analyses showed that there was no carry-over effect of first period to second period treatment. Finally, the effect of possible confounders, such as smoking status and previous inhaled steroid usage, on roflumilast treatment could not be assessed, due to the limited sample size. In order to investigate these effects, as well as examine whether the anti-inflammatory effect of roflumilast is causally related to the improvement in lung function, prospective trials with larger sample size should be performed.

How can the present results be explained? Roflumilast is a new oral, once-daily administered inhibitor targeting PDE4. In patients with COPD, systemically available treatment may interact with pulmonary as well as systemic inflammation, both of which are present in COPD (1;4). Therefore, systemic effects of roflumilast associated with the initiation of inflammation can be anticipated. TNFα is a major product of mononuclear leukocytes in the circulation, which is regulated by cAMP (15). TNFα is involved in upregulation of the adhesion molecule E-selectin on vascular endothelium (30). Selectin-mediated adhesion of leukocytes to the vascular endothelium, is a key early event in the initiation of inflammatory responses (30).
Interestingly, elevated levels of E-selectin in serum have been found in COPD patients (9). In the present study, PDE4 inhibition by roflumilast was associated with a slight reduction in TNFα release following ex vivo stimulation of whole blood cells. However, E-selectin levels in serum were unchanged during roflumilast treatment. This may indicate that either the reduction in TNFα was too small to exert an effect on the expression of adhesion molecules, or that ex vivo releasability of TNFα by whole blood cells does not predict E-selectin release.

Microvascular leakage is regulated through the formation and closure of intercellular gaps between endothelial cells, which facilitate the extravasation of fluid, macromolecules and cells (31). Elevation of cAMP decreases intercellular gap formation and permeability, thereby restoring the pulmonary endothelial barrier integrity (31). In pulmonary endothelial monolayers cultured in vitro, induced hyperpermeability can be reduced by PDE3 and PDE4 inhibitors (32). The finding that α2-macroglobulin levels in sputum supernatant, a marker of microvascular leakage (33), were reduced by roflumilast, suggest that also in vivo inhibition of PDE4 leads to restoration of the endothelial barrier. Apparently, this resulted in non-differential attenuation of cell extravasation from the circulation, as shown by a reduction in absolute cell counts for neutrophils, eosinophils, macrophages and lymphocytes in sputum without an effect on cell differentials. These effects are not observed only in sputum samples, but also in bronchial biopsy specimens (26).

Results of several studies have shown that the level of inflammation within the airways is associated with lung function in patients with COPD (34;35). It is likely that attenuation of airways inflammation by roflumilast is one of the mechanisms underlying the observed improvement in lung function. Although a direct effect of PDE4 inhibition on smooth muscle relaxation cannot be excluded (12), this does not seem a likely explanation for the observed improvement in lung function because it
has been shown that a single dose of PDE4 inhibitor does not provide bronchodilation (28;36).

Extensive in vitro and in vivo studies in animals have demonstrated that inhibition of PDE4 increases the intracellular concentration of cAMP, leading to broad anti-inflammatory and immunomodulatory effects (15;16). Indeed, in our study, the activity of several types of inflammatory cells was suppressed following roflumilast, as shown by large reductions in the levels of IL-8, neutrophil elastase and eosinophil cationic protein. Although lactoferrin levels were not significantly reduced by roflumilast, the effect size was in the same order as for the other soluble markers. Alternatively, it could be speculated that PDE4 inhibition selectively affects the degranulation of azurophilic granules in neutrophils, containing neutrophil elastase (37), rather than the specific granules, containing lactoferrin (37). Because not only neutrophils, but also the submucosal glands are a major cellular source of lactoferrin production, an inhibitory effect of treatment on lactoferrin release by neutrophils may have been masked.

Our data have important clinical implications. At present, the recommended treatment of COPD consists of bronchodilators for symptom relief, with addition of inhaled corticosteroids for more severe disease (1;19). Inhaled steroids have only limited effects on airway inflammation and lung function (decline) in patients with COPD (10;38;39). Roflumilast treatment in COPD improves not only lung function and health status (18), but also reduces the rate of mild exacerbations as judged from an increase in bronchodilator use and symptoms (18). This may be associated with reduced airway inflammation, as observed in our study.

In summary, our results support the hypothesis that roflumilast treatment has anti-inflammatory effects in patients with COPD. Whether such treatment also reduces
airway remodelling, the progressive decline in FEV₁ and the associated mortality risk in patients with COPD remains to be examined in long-term follow-up studies.
Acknowledgement

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Competing interest statement

Diana Grootendorst, Stefanie Gauw and Renate Verhoosel have no declared conflict of interest. The Department of Pulmonology, and thereby Peter Sterk (staff member), Pieter Hiemstra (staff member) and Klaus Rabe (head of the department) has received grants from AltanaPharma (222.616 US $), Novartis (90.640 US $), Bayer (61.762 US $), AstraZeneca (113.155 US $), Pfizer (406.000 US $), MSD (118.000 US $), Exhale Therapeutics (90.000 US $), Boehringer Ingelheim (390.000 US $), Roche (120.000 US $) and GSK (299.495 US $) in the years 2001 until 2006. Pieter Hiemstra has participated as a speaker in various meetings co-financed by various pharmaceutical companies. Klaus Rabe has been consulting, participated in Advisory Board Meetings and received lecture fees from AstraZeneca, AltanaPharma, MSD and GSK. Klaus Rabe holds no stock or other equities in pharmaceutical companies. Jeannette Hospers, Dirk Bredenbröker, and Thomas Bethke are employees of ALTANA.

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which was subsequently discussed with and approved by ALTANA. All investigators and ALTANA agreed upon submission of the manuscript to Thorax.
Table 1. Demographics of patients in the efficacy analysis.

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Gender (m:f)*</td>
<td>29:9</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>63.1 (7.0)</td>
</tr>
<tr>
<td>Smoking history (pack yr)*</td>
<td>40.0 (10-90)</td>
</tr>
<tr>
<td>Smoking status (current:ex)*</td>
<td>18:20</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV$_1$ (L)</td>
<td>1.79 (0.60)</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV$_1$ (%predicted)</td>
<td>57.9 (12.9)</td>
</tr>
<tr>
<td>Post-bronchodilator FEV$_1$ (%predicted)</td>
<td>61.0 (12.6)</td>
</tr>
<tr>
<td>Reversibility (% from pre-bronchodilator FEV$_1$)</td>
<td>6.0 (6.0)</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV$_1$/FVC (%)</td>
<td>54.4 (10.1)</td>
</tr>
</tbody>
</table>

Data are mean (SD), *number, or #median (minimum-maximum).
Table 2. Inflammatory markers in sputum and blood at baseline of patients in the efficacy analysis.

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>x10^5 cells/g sputum</th>
<th>concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Induced sputum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total non-squamous cells</td>
<td>-</td>
<td>18.0 (2.0-87.1)</td>
<td></td>
</tr>
<tr>
<td>Squamous cells</td>
<td>10.7 (0-74.4)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>74.4 (46.4-95.4)</td>
<td>13.0 (1.7-84)</td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>20.5 (2.6-47.6)</td>
<td>3.2 (0.2-21.1)</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.8 (0.02-8.0)</td>
<td>0.14 (0.001-4.2)</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.4 (0-4.6)</td>
<td>0.24 (0.024-1.87)</td>
<td></td>
</tr>
<tr>
<td><strong>Markers in sputum supernatant</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-8 (ng/mL)</td>
<td></td>
<td>12.5 (0.49-179.9)</td>
<td></td>
</tr>
<tr>
<td>Neutrophil elastase (µg/mL)</td>
<td></td>
<td>3.61 (0.14-120.3)</td>
<td></td>
</tr>
<tr>
<td>Lactoferrin (µg/mL)</td>
<td></td>
<td>73.5 (0.8-512.9)</td>
<td></td>
</tr>
<tr>
<td>ECP (µg/L)</td>
<td></td>
<td>170.8 (15.6-5767.5)</td>
<td></td>
</tr>
<tr>
<td>α_2-macroglobulin (ng/mL)</td>
<td></td>
<td>1345.7 (20.1-15063.04)</td>
<td></td>
</tr>
<tr>
<td><strong>Blood</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-selectin (ng/mL)</td>
<td></td>
<td>51.7 (33.5-94.6)</td>
<td></td>
</tr>
<tr>
<td>TNFα (ng/mL)</td>
<td></td>
<td>8.1 (1.6-37.0)</td>
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</tbody>
</table>

Data are median or *geometric mean (minimum-maximum).
Table 3. Mean overall difference between roflumilast and placebo treatment.

<table>
<thead>
<tr>
<th>Sputum characteristics</th>
<th>Mean difference (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell count (x10^5 cells/gram)*</td>
<td>-33.6 % (-48.8, -13.8)</td>
<td>0.0023</td>
</tr>
<tr>
<td>Weight (g)*</td>
<td>0.15 (-0.56, 0.86)</td>
<td>0.68</td>
</tr>
<tr>
<td>Viability (%)*</td>
<td>-2.0 (-4.4, 0.3)</td>
<td>0.091</td>
</tr>
<tr>
<td>Squamous cell contamination (%)*</td>
<td>-1.8 (-6.0, 2.5)</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Cellular inflammation in sputum

| Absolute number of neutrophils*                                                                  | -35.5 % (-50.7, -15.6) | 0.0017 |
| Absolute number of eosinophils*                                                                 | -50.0 % (-65.8, -26.8)  | 0.0005 |
| Absolute number of macrophages*                                                                  | -24.4 % (-43.9, 2.0)    | 0.067  |
| Absolute number of lymphocytes*                                                                  | -34.8 % (-54.7, -6.1)   | 0.022  |
| % Neutrophils*                                                                                    | -1.6 (-4.8, 1.6)        | 0.31   |
| % Eosinophils*                                                                                   | -25.2 % (-44.3, 0.3)    | 0.052  |
| % Macrophages*                                                                                   | 1.8 (-0.9, 4.6)         | 0.19   |
| % Lymphocytes*                                                                                   | -4.1 % (-23.8, 20.7)    | 0.72   |

Markers of inflammation in sputum supernatant

| IL-8 (ng/mL)*                                                                                     | -25.9 % (-44.7, -0.8)   | 0.044  |
| Neutrophil elastase (µg/mL)*                                                                     | -30.6 % (-49.8, -4.1)   | 0.028  |
| Lactoferrin (µg/mL)*                                                                             | -29.0 % (-57.3, 17.9)   | 0.18   |
| ECP (µg/L)*                                                                                      | -34.3 % (-53.1, -7.9)   | 0.015  |

Markers of microvascular leakage in sputum supernatant

| α2macroglobulin (ng/mL)*                                                                          | -40.8 % (-55.0, -22.3)  | 0.0003 |

Systemic markers of inflammation

| E-selectin (ng/mL)*                                                                                | -1.7 % (-4.5, 1.1)       | 0.23   |
| TNFα (pg/mL)*                                                                                    | -10.4 % (-19.7, -0.1)    | 0.047  |

Lung function

| Pre-bronchodilator FEV₁ (mL)*                                                                     | 79.5 mL (54.0, 105.1)    | <0.0001|
| Post-bronchodilator FEV₁ (mL)*                                                                   | 68.7 mL (12.9, 124.5)    | 0.018  |

*Mean difference between treatments represents ‘absolute’ difference between Roflumilast and Placebo treatment for sputum weight, viability, squamous cell contamination, % neutrophils and % macrophages in sputum, and pre- and post-bronchodilator FEV₁, and ‘relative’ difference (mean ratio of change from baseline) for total cell count, number of neutrophils, eosinophils, macrophages and lymphocytes, %eosinophils, % lymphocytes, IL-8, neutrophil elastase, lactoferrin,
ECP, \( \alpha_2 \)macroglobulin, E-selectin and TNF\( \alpha \) since these parameters were log-transformed prior to analyses; \( ^{\dagger} \)unit of absolute cells in sputum: \( x10^5 \) cells per gram sputum.
Table 4. Patients (%) with adverse events during treatment periods.

<table>
<thead>
<tr>
<th>System</th>
<th>Roflumilast</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body as a whole (flu syndrome, abdominal pain, malaise, back pain etc.)</td>
<td>17 (45)</td>
<td>10 (26)</td>
</tr>
<tr>
<td>Digestive system (diarrhea, nausea, abnormal stool, vomiting, dyspepsia etc.)</td>
<td>17 (45)</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Nervous system (headache, dizziness)</td>
<td>17 (45)</td>
<td>2 (5)</td>
</tr>
<tr>
<td>Respiratory system (increased cough, dyspnea, upper respiratory tract infection, rhinitis, increased sputum production)</td>
<td>16 (42)</td>
<td>15 (39)</td>
</tr>
<tr>
<td>Cardiovascular system (ECG abnormal, chest pain, palpitation, thrombophlebitis)</td>
<td>4 (11)</td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

Adverse events with incidence greater or equal to 1% are listed.
Legends to the figures.

**Figure 1.** Following a 2-week run-in period, patients were randomized to receive roflumilast (500 µg once daily) or matching placebo for a period of 4 weeks. Four to 6 weeks after wash-out of the medication of the first treatment period, patients crossed over to the alternative treatment. During each treatment period, patients visited the department on a weekly basis. LF: lung function measurement by spirometry, rev: reversibility testing of FEV₁ with 400 µg salbutamol.

**Figure 2.** Change in pre- and post-bronchodilator FEV₁ during roflumilast (black bars) and placebo (white bars) treatment for 4 weeks.

**Figure 3.** Change in total cell count, number of neutrophils and eosinophils in sputum during roflumilast (black bars) and placebo (white bars) treatment for 4 weeks.

**Figure 4.** Change in ECP, IL-8 and neutrophil elastase, lactoferrin and α₂-macroglobulin levels in sputum supernatants during roflumilast (black bars) and placebo (white bars) treatment for 4 weeks.
Reference List


(13) Lipworth BJ. Phosphodiesterase-4 inhibitors for asthma and chronic obstructive pulmonary disease. Lancet 2005 Jan 8;365(9454):167-75.


