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ORIGINAL RESEARCH

Roflumilast, a phosphodiesterase-4 inhibitor, induces phagocytic activity in Greek COPD patients

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Background: A new approach to the treatment of COPD includes controlling inflammation because of its important role in exacerbation of the disease. Recently, roflumilast has been added as a therapeutic option for COPD. Roflumilast is an oral phosphodiesterase-4 inhibitor that targets inflammatory cells involved in triggering exacerbations of COPD. The objective of the current study was to evaluate roflumilast for its contribution to phagocytic activity in COPD patients.

Methods: Twenty-one patients diagnosed with COPD received roflumilast once daily for 6 months in combination with fluticasone (an inhaled corticosteroid), salmeterol (a long-acting β2-agonist), and tiotropium (a long-acting muscarinic antagonist) or combinations of these agents. The main inclusion criterion was stable disease for at least the previous 30 days. Neutrophils and spirometric changes, ie, forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC), were measured in the COPD patients at indicated time points. The first sample was taken before receiving roflumilast, the second 3 months later, and the third after 6 months. Examination of defective phagocytosis was done by flow cytometry using a FagoFlowEx® kit. The statistical analysis was performed using Statistica software.

Results: Our results indicate that phagocytic activity was increased after 3 and 6 months of treatment when compared with baseline ($P<0.001$). Similarly, FVC and FEV_1 were also increased during the 6-month period, but only FVC differed significantly from baseline ($P<0.001$).

Conclusion: Although the number of patients in this study was limited, our results indicate that roflumilast induces phagocytic activity, which improves lung function.

Keywords: COPD, roflumilast, phosphodiesterase-4 inhibitors

Introduction

COPD is a chronic inflammatory disease affecting the lungs. Cigarette smoking is the most important risk factor, although smoke derived from burning biomass fuels is also a predisposing factor in developing countries.[1,2]

Diagnosis of COPD is confirmed by spirometry, ie, a post-bronchodilator forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC, Tiffeneau index) of 0.7. COPD is referred to as severe when the FEV_1 is 50%–30% and very severe when it is <30% of the predicted value.[3] Recent global guidelines for the management of COPD recommend grouping patients into four groups labeled A, B, C, or D according to symptoms (classified as "less" and "more"), air flow limitation (classified as "mild", "moderate", "severe", and "very severe", Global Initiative for Chronic Obstructive Lung Disease stages 1–4 respectively), risk of exacerbation (classified as "low"

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[≤1 exacerbation per year] and "high" [≥2 exacerbations per year]), and the presence of comorbidities.[4]

Typical pathological changes in COPD include destruction of the lung parenchyma, eg, emphysema and obstructive bronchiolitis, characterized functionally by progressive airway obstruction. Chronic cough and hypersecretion of mucus may also occur due to inflammatory changes and hyperplasia of the mucous glands in the larger airways.[5] The clinical course of COPD is characterized by acute exacerbations accompanied by acute deterioration in lung function and worsening disability.[6]

The predominant inflammatory cells in COPD are CD68 macrophages and CD8 T-lymphocytes, with polymorphs increasing during acute exacerbations. The severity of inflammation increases during worsening of COPD, resulting in luminal narrowing, parenchymal destruction, and diminishing elastic recoil. The activated macrophages release inflammation mediators and chemotactic factors, including proinflammatory cytokines such as tumor necrosis factor-alpha and interleukin (IL)-6, IL-8, monocyte chemotactic peptide-1, leukotriene B4, and reactive oxygen species, and secrete proteolytic enzymes, in particular matrix metalloproteinases 9 and 12.[7] Neutrophils move directly to the respiratory tract under the influence of secreted chemotactic factors (particularly IL-8 and leukotriene B4) and cause overstimulation of submucosal mucous glands and goblet cells by proteinases, especially neutrophil elastase, cathepsin-G, and proteinase-3.[8]

Although care of COPD has improved over the last decade,[9] there is still a huge unmet burden of disease. Exacerbations in particular can be distressing for patients[10] and have serious consequences with regard to their long-term health, such as increased disease progression,[11] increased risk of cardiovascular events,[12] and increased mortality rates.[13,14]

In clinical practice, patients still suffering from frequent exacerbations may already be treated with a combination of a long-acting β2-agonist (LABA) and inhaled corticosteroids (ICS), often in addition to tiotropium (a long-acting muscarinic antagonist [LAMA]). Despite the wide variety of pharmacological treatments currently available for COPD, they are insufficient to prevent exacerbations in a number of patients with severe to very severe disease. Since frequent exacerbations are associated with a high level of inflammation,[15,16] it is logical to add an anti-inflammatory therapy to combination treatment in order to further reduce the risk of exacerbation.

Roflumilast is an oral phosphodiesterase (PDE)-4 inhibitor that targets inflammatory cells involved in triggering exacerbations of COPD. It is the only PDE-4 inhibitor approved by the US Food and Drug Administration and is available in 500 μg tablets for once-daily administration. It is catalyzed by cytochrome P450 1A2 and 3A4 to its active metabolite, roflumilast N-oxide, which is responsible for >90% of the total PDE-4 inhibitory activity of roflumilast.[17]

Eleven distinct PDE families have been identified, although most of the anti-inflammatory activity is believed to result from inhibition of PDE-4, for which there is clinical correlation. Three critical findings contributed to the rationale for developing selective PDE-4 inhibitors. First, PDE-4 regulates degradation of 3′5′-cyclic adenosine monophosphate in most immune and proinflammatory cells; second, in cell-based systems, PDE-4 inhibitors of varied structural classes suppress a plethora of responses that are considered to be proinflammatory; and third, PDE-4 inhibitors are efficacious in preclinical animal models that attempt to reproduce specific facets of the pathobiology of COPD.[18] Further, PDE-4 inhibitors ameliorate the activity of various inflammatory cells in vitro[19] and reduces pulmonary inflammation in complex in vivo animal models.[20,21] Specifically, the pathobiological aspects of COPD where PDE-4 inhibitors are efficacious have been reported in: in vitro studies, such as decreased apoptosis (which may result in clearance of sputum), decreased release of cytokines in many cell types and release of inflammatory mediators in neutrophils;[22] in vivo studies, such as inhibition of cell trafficking, and release of cytokines and chemokines from inflammatory cells such as neutrophils, eosinophils, macrophages, and T-cells;[23] and in animal models, such as decreased accumulation of neutrophils in bronchoalveolar lavage fluid or abolition of the influx of inflammatory cells into the lung parenchyma.[24]

Clinical trials have already demonstrated the ability of roflumilast to decrease the frequency of exacerbations and improve lung function in COPD,[25,26] while its biological action may result potentially in targeting of the inflammatory processes underlying COPD. Thus, it is recognized that the inflammatory response of the lungs is an important field of research necessary for understanding the disease process and for subsequent development of novel therapies for COPD.

Given that the inflammatory infiltrate found in the airway lumen in patients with COPD consists mainly of neutrophils,[27] the objective of this open-label study was to evaluate roflumilast, a PDE-4 inhibitor, for its contribution to the phagocytic activity of neutrophils in patients with COPD and its therapeutic potential in terms of diminishing the inflammatory response associated with the disease. Thus, our hypothesis was that addition of an anti-inflammatory regimen such as roflumilast in patients who experience frequent exacerbations would have a beneficial influence on lung function and exacerbations.

Materials and methods

Ethics

Our study was approved by the investigational review board of G Papanikolaou General Hospital, Thessaloniki, Greece.

Treatment regimens

In our study, roflumilast was administered to COPD patients as 500 μg tablets once daily in addition to other COPD treatments, including fluticasone (an ICS), salmeterol (an LABA), and tiotropium (an LAMA).

Patients

Twenty-one patients diagnosed with severe or very severe COPD (groups C and D) with a bronchitis phenotype received roflumilast once daily for 6 months or more in combination with other COPD treatments (including an LABA + ICS + LAMA or an LABA + ICS; Table 1). Entry criteria of patients included stable disease for at least 30 days. All patients had a history of one or two exacerbations during the previous year, but none were recovering from an acute exacerbation. All patients were ex-smokers and, to the best of our knowledge, no patient was smoking during the study period.

Spirometry

FEV_1 and FVC were measured at indicated time points, ie, baseline, 3 months, and 6 months.

Samples

Peripheral blood samples were taken from the COPD patients at the indicated time points. The first sample was taken before receiving roflumilast, the second after 3 months, and the third after 6 months of treatment.

Flow cytometry

Examination of phagocytosis was done by flow cytometry using the FagoFlowEx® kit (Exbio Diagnostics, Ramona, CA, USA). This kit enables examination of the phagocytic activity of granulocytes in heparinized whole blood by measuring the respiratory (oxidative) burst after their stimulation with inactive *Escherichia coli* bacteria.

Table 1 Patient characteristics

Characteristics	Patients (n=21)
Mean age, years	67.4
Sex (male, female)	(86%, 14%)
Group (C, D)	(72%, 28%)
LABA + ICS + LAMA, LABA + ICS	(28%, 72%)
Frequency of exacerbations (1, 2)	(80%, 20%)
FEV_1 %pred (mean)	42.3%
FVC %pred (mean)	63.5%

Abbreviations: ICS, inhaled corticosteroids; LABA, long-acting β2-agonist; LAMA, long-acting muscarinic antagonist; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; %pred, percent predicted.

Statistical analysis

The statistical analysis was performed using Statistica software (StatSoft Inc., Tulsa, OK, USA). Means calculated from statistically significant factors were examined for potential differences between factor levels by comparing their 95% confidence intervals based on the pooled standard error of analysis of variance. Intervals that do not overlap indicate means that differ significantly from each other. In all statistical analyses, the 0.05 probability level of significance was chosen as the level of reference.

Results

Our results indicate that phagocytic activity was increased during the 6-month treatment period when compared with the first sample. The mean dependence of phagocytic activity before receiving roflumilast differs significantly from the rest periods, since the 95% confidence intervals do not overlap, bringing out a particular pattern of change ($0<3$, $3>6$; Figure 1). Similarly, FVC and FEV_1 values were also increased at the 3-month and 6-month time points when compared with the first sample. However, only FVC values differed significantly. The mean dependence of FVC values before receiving roflumilast differed significantly from the rest periods ($P<0.001$, Table 2).

Discussion

The last Global Initiative for Chronic Obstructive Lung Disease publication recommended a new staging system that contains the frequency of exacerbations,[4] and treatment recommendations are made taking into account the group into which the patient falls.

In 2010, roflumilast, a selective PDE-4 inhibitor with anti-inflammatory properties, was approved by the European Medicines Agency for use as once-daily oral maintenance treatment for adult patients with severe or very severe COPD associated with chronic bronchitis and a history of frequent exacerbations, as an add-on to bronchodilators. The hypothesis was that roflumilast provides a large benefit to a subset of COPD patients due to its ability to block activation of inflammatory cells. In our study, phagocytic activity was increased, and this anti-inflammatory effect may in part explain the concomitant improvement in spirometry values, as indicated by our results.

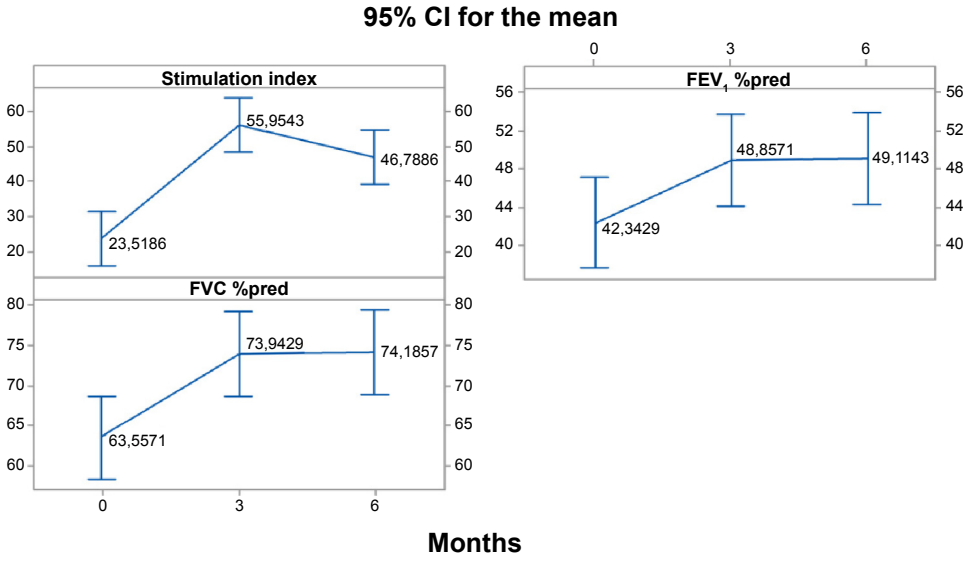

Figure 1 Mean change in stimulation index for phagocytic activity, FEV_1 %pred, FVC %pred, per indicated periods (0, before receiving roflumilast, and 3 months and 6 months after).
Notes: Vertical axes represent the 95% confidence intervals of means calculated from the mean squared error of analysis of variance. The pooled standard deviation was used to calculate the intervals.
Abbreviations: CI, confidence interval; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; %pred, percent predicted.

Neutrophils play a central role in COPD, and their accumulation and degranulation are associated with tissue damage, increased inflammation, and disordered tissue repair. In particular, the effect of roflumilast on neutrophils is possibly due to the increase in intracellular 3′5′-cyclic adenosine monophosphate attributable to PDE-4 inhibition.[28] Furthermore, PDE inhibitors decrease neutrophil damage.[29]

Until recently, it was assumed that the activity and function of neutrophils was a response to the presence of high levels of inflammation in the lung. However, more recent studies of neutrophil function (including migration, generation of reactive oxygen species, degranulation, phagocytosis, and extracellular trap production) suggest that there is a general impairment of neutrophil responses in COPD that predisposes to increased inflammation and reduced clearance of bacteria.[1] This could be very useful for restoring some key neutrophil responses in COPD. Targeting neutrophil intracellular signaling might improve disease outcomes by reducing the extraneous inflammatory burden.

In our study, the FVC in COPD patients was increased significantly in the first 3 months after starting roflumilast in combination with other therapy regimens, such as LABA + ICS + LAMA and LABA + ICS. FEV_1 was not significantly improved. This is possibly due to the limited number of patients in our study or perhaps due to the short time period examined. Our results also show that FVC and FEV_1 values not only increased during the first 3 months but also remained stable during the following 3 months (Figure 1). These findings are in accordance with several other studies.[29,30] PDE-4 inhibition by roflumilast treatment for 4 weeks improved post-bronchodilator FEV_1, and at the same time resulted in an anti-inflammatory effect whereby the numbers of neutrophils and eosinophils were reduced, as well as soluble markers of neutrophilic and eosinophilic inflammatory activity in induced sputum samples of patients with COPD.[30]

Other clinical investigators have reported similar results. Treatment with a PDE-4 inhibitor was associated with a significant improvement in FEV_1 over the trial period compared with placebo in patients with COPD (15 trials, 12,654 patients).[31] Similarly, in clinical trials of Asian COPD patients, roflumilast provided a sustained increase in mean prebronchodilator and post-bronchodilator FEV_1 and in prebronchodilator and post-bronchodilator FVC when compared with placebo.[32,33] The investigators concluded that roflumilast is an optimal treatment choice for patients with severe to very severe COPD. In another study (n=3,091), roflumilast reduced exacerbation rates and improved lung function in patients with COPD who received a concomitant LABA, regardless of prior ICS use, and across various

Table 2 *P*-values from statistical analysis

Analysis of variance	
Stimulation index	0.000
FEV_1 %pred	0.088
FVC %pred	0.007

Abbreviations: FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; %pred, percent predicted.

patient subgroups regardless of age and smoking status.[34] In a meta-analysis of eleven trials involving 9,675 patients, roflumilast significantly reduced the mean exacerbation rate (mild, moderate, or severe) and improved trough FEV_1, FVC, and other post-bronchodilator spirometric parameters.[35] In six placebo-controlled trials involving nearly 4,500 patients with COPD of varying severity, use of roflumilast was associated with reduced COPD exacerbations and improved lung function, as determined by spirometry, with the greatest benefits observed in patients with severe COPD who had chronic bronchitis and a history of frequent exacerbations; clinical efficacy was demonstrated in patients receiving roflumilast alone and in those receiving LABA therapy.[36]

There has been a number of reports concerning the adverse effects of roflumilast. In clinical trials, the most common adverse events reported were diarrhea, nausea, and headache. Weight loss and an increased risk of psychiatric events have also been reported in association with roflumilast. In our study, six (28%) patients reported diarrhea and four (19%) reported headache, but no patient wished to be withdrawn from treatment with roflumilast.

Management of stable COPD should focus on reducing the patient's future risk of disease progression (determined primarily by exacerbation frequency).[37] We should also take into account systemic inflammation as the biological link in combination with exacerbations for evaluation of COPD severity. According to the guidelines, use of combinations of all these treatments is recommended when the severity of COPD increases.[3] However, until now, no therapy has been shown to treat the underlying inflammation found in COPD.

To the best of our knowledge, this is the first study in which phagocytic activity was measured in COPD patients receiving roflumilast. A limitation of our study is the small number of patients included; however, our study is an ongoing pilot trial. Future studies should include biomarkers of systemic inflammation and cytokines, and will shed more light on improvement in lung function and reduction of exacerbations.

In conclusion, in our study, treatment with roflumilast induced phagocytic activity which improved lung function. We should view COPD as a preventable and treatable disease. As roflumilast has novel anti-inflammatory activity in COPD, it provides the physician with a treatment option beyond bronchodilation. Although this was a small study, the anti-inflammatory activity of roflumilast was shown to provide incremental benefits on top of existing therapies. Future randomized studies will further confirm the impact of roflumilast on COPD.

Disclosure

An abstract of this paper appeared in a supplement to the *Journal of Thoracic Disease* containing abstracts presented at the Pan Hellenic Congress on COPD in 2014. Otherwise, the authors report no conflicts of interest in this work.

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