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Effect of 6 Months of Erythromycin Treatment on Inflammatory Cells in Induced Sputum and Exacerbations in Chronic Obstructive Pulmonary Disease

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For editorial comment see p. 441

Key Words
Airway inflammation · Anti-inflammatory effects · Chronic obstructive pulmonary disease · Exacerbation, respiratory · Macrolides · Neutrophils

Abstract

Background: Chronic obstructive pulmonary disease (COPD) is characterized by airway inflammation and is associated with acute exacerbations. Macrolide antibiotics have been shown to exhibit anti-inflammatory effects in some chronic airway inflammatory diseases. ***Objective:*** The aim of this study was to assess the effect of treatment with erythromycin on airway inflammation and health outcome in COPD patients. ***Methods:*** We conducted a randomized, placebo-controlled, double-blind trial of erythromycin for a period of 6 months. Thirty-six COPD patients were randomized to treatment with oral erythromycin (125 mg, three times/day) or placebo. The primary outcomes were neutrophil number in sputum and exacerbations. ***Results:*** Thirty-one patients completed the study. At the end of treatment, neutrophil counts in the sputum were significantly decreased in the group treated with erythromycin compared with placebo-treated patients ($p = 0.005$). Total cells in the sputum and neutrophil elastase in sputum supernatant were also significantly decreased in those treated with erythromycin compared with the placebo group ($p = 0.021$ and $p = 0.024$, respectively). The mean exacerbation rate was lower in the erythromycin group than in the placebo group (relative risk = 0.554, $p = 0.042$). Kaplan-Meier survival analysis showed that erythromycin significantly delayed the time to the first COPD exacerbation compared with placebo ($p = 0.032$). ***Conclusions:*** Erythromycin treatment in COPD patients can reduce airway inflammation and decrease exacerbations and may therefore be useful in the management of COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common cause of morbidity and mortality worldwide [1–3]. It is characterized by persistent pulmonary inflamma-

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tion, which is thought to lead to progressive airflow limitation. Exacerbations of COPD are associated with increased lung inflammation and impair in the quality of life of patients [4]. Thus therapeutic interventions to reduce lung inflammation may lead to improved outcome in COPD patients.

Airway inflammation in COPD can be assessed by examination of cells and inflammatory mediators in induced sputum [5]. Smokers and exsmokers with COPD have increased sputum neutrophil numbers compared with subjects without COPD, which increase further during exacerbations. Increased sputum neutrophils have been associated with a more rapid decline in forced expiratory volume in 1 s (FEV_1) [6–8]. Furthermore, neutrophil activation markers, e.g. neutrophil elastase (NE), myeloperoxidase and lactoferrin, are elevated in sputum supernatants of COPD subjects [9], suggesting that neutrophils are active participants in airway inflammation.

Inflammation in COPD lungs is largely resistant to therapy with corticosteroids and consequently alternative approaches of anti-inflammatory therapy are needed [10, 11]. Long-term treatment with macrolides is an accepted and effective treatment in some chronic lung diseases [12], especially for diffuse panbronchiolitis (DPB) [13]. Beneficial effects of macrolides have also been shown in other airway inflammatory diseases such as cystic fibrosis [14, 15], bronchiectasis [16], and bronchial asthma [17, 18]. The mechanisms by which macrolides exert a beneficial effect on chronic inflammatory airway disease are thought to be independent of their antibiotic effects but rather due their anti-inflammatory effects. These include inhibition of cytokine production by neutrophils, monocytes and bronchial epithelial cells [19–21]. Treatment with erythromycin has been shown to reduce the number of neutrophils and the levels of interleukin-8 (IL-8) protein in bronchoalveolar lavage fluid from patients with DPB and cystic fibrosis [10, 21–24]. We and others have shown that the anti-inflammatory effects of erythromycin and clarithromycin are associated with inhibition of the transcription factors activator protein-1 and nuclear factor-κB [25, 26].

The effects of macrolide treatment on lung inflammation in COPD patients have not been studied. We therefore conducted a randomized, double-blind, placebo-controlled trial of erythromycin (125 mg three times a day) in COPD patients to assess its effects on sputum cell counts and health outcome in COPD patients.

Materials and Methods

Patients

We recruited patients meeting the GOLD diagnostic criteria for COPD [27]. Patients were ≥40 years old; had a diagnosis of COPD, an FEV_1 between 30 and 70% of predicted, FEV_1/(forced vital capacity) FVC <0.7, and FEV_1 reversibility <15% and/or <200 ml to β_2-agonists, and were past or present cigarette smokers with at least a 10 pack-year smoking history. Patients were studied when clinically stable for at least 1 month following an exacerbation. Patients with significant respiratory disease other than COPD were excluded. Patients were also excluded if there was a history of unstable cardiovascular disorders or hypersensitivity to macrolides. During the study period, no change in any therapy with anti-inflammatory activity was allowed unless there was clinical necessity, in which case the patient was excluded from the study. Patients gave written informed consent to participate, and the study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University.

Study Design

Patients had a 1-month run-in period free of exacerbation symptoms before baseline sampling. Eligible participants were randomly assigned to receive oral erythromycin (125 mg three times a day, enteric-coated tablets; DaLian Metro Pharmaceutica, China) or placebo for 6 months. Spirometry, quality of life, exacerbations, and sputum assessment for cell counts and inflammatory markers were recorded at 0 (baseline), 3 and 6 months. The co-primary outcomes for this study were sputum neutrophil numbers and exacerbations. Treatment adherence was encouraged by calls from the study coordinator and measured by pill counts.

Analysis of Sputum Samples

Induced sputum was performed according to a standard technique, as previously described [5]. The collected sputum was dispersed using dithiothreitol. Total and differential counts of inflammatory cells were performed and sputum supernatant was stored. The NE level in the supernatant of induced sputum was assessed by enzyme-linked immunosorbent assay kits (Human PMN-Elastase ELISA, Bender MedSystems, Austria). Sputum bacterial culture was performed at baseline and after 6 months of treatment.

Quality of Life

Health status was measured using the SGRQ and the Short Form 36-item Questionnaire (SF-36) at baseline, and after 3 and 6 months [28, 29].

Monitoring and Definition of Exacerbation

The severity of an exacerbation was graded as follows: a moderate exacerbation was defined as a sustained worsening of baseline respiratory symptoms for at least 2 days that required increased treatment or additional therapy such as oral corticosteroids or antibiotics. A severe exacerbation was defined as above but with the requirement for admission to hospital. Information on exacerbations was collected during clinic visits, and any patient experiencing worsening respiratory symptoms was instructed to contact the investigator immediately and report to the study clinic as soon as possible. Exacerbation frequency was taken as the

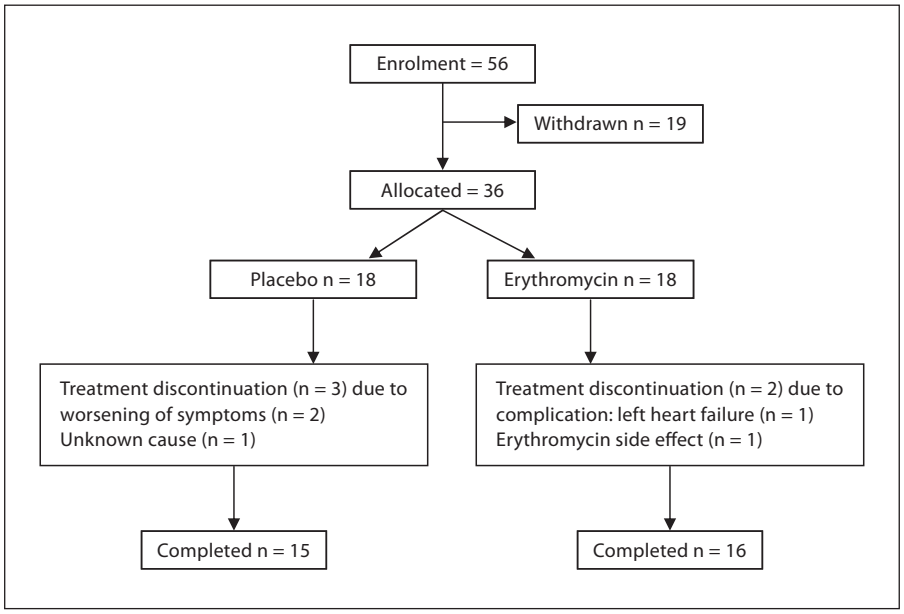

Fig. 1. Study profile: all patients were entered into the intent-to-treat analysis.

number of moderate-severe exacerbations over the 6-month follow-up period.

Monitoring Adverse Events

At entry and trial completion, a 12-lead electrocardiograph, blood pressure measurements and physical examination were performed. Routine biochemistry and hematology testing were also assessed at study entry, and after 3 and 6 months. The presence of adverse events, including the presence of fever, headache, nausea, vomiting, diarrhea and skin rashes, was recorded at each study visit.

Statistical Analysis

The final analysis was conducted on an intention-to-treat basis using SPSS software (version 16.0). Data were expressed as means, standard deviations and 95% confidence intervals unless otherwise stated. A p value <0.05 was considered significant (two-tailed tests).

Comparison of the baseline data between both groups was analyzed using the two-sample t test. Serial stable lung function, total cells and NE in sputum, and SGRQ and SF-36 data at baseline, and after 3 and 6 months were compared by linear mixed-model analysis with visit number as the repeated measure. A similar linear mixed model was used to examine differences between erythromycin and placebo groups.

The number of exacerbations was analyzed using a generalized linear model for a Poisson distribution (expressed as mean rate, i.e. mean number of exacerbations per patient in 6 months). The number of moderate-severe exacerbations was used as the dependent outcome variable, using the logarithm of time on treatment as an offset variable and the smoking index, sex, percent of predicted FEV_1 at baseline and age as covariates. The time to first exacerbation between the placebo and macrolide groups was analyzed by Kaplan-Meier survival analysis.

Results

Fifty-five patients were screened for the study, and 36 were eligible for study entry (fig. 1). Of these 36 patients, 18 were assigned to receive placebo and 18 were assigned to erythromycin. Thirty-one of the randomized patients completed the study; 3 in the placebo group and 2 in the erythromycin group withdrew (fig. 1).

Demographic Data

There were no significant differences between both study groups at baseline with respect to age, gender, lung function, smoking history and body mass index. Virtually all patients received at least one respiratory medication, and respiratory medication use was similar in the two treatment groups (table 1).

Sputum Cell Counts and NE

Baseline total sputum counts and differential cell counts were similar between the two groups (table 2). Treatment with erythromycin significantly decreased the total number of cells from baseline to months 3 and 6 ($p = 0.005$ and $p = 0.004$, respectively). This reduction was also significantly greater compared with placebo ($p = 0.021$). Erythromycin also produced a similar reduction in neutrophil cell counts from baseline to months 3 and 6 ($p = 0.002$ and $p = 0.001$, respectively; table 2). This decrease was significantly different compared with placebo treatment ($p = 0.005$). In contrast, the number of

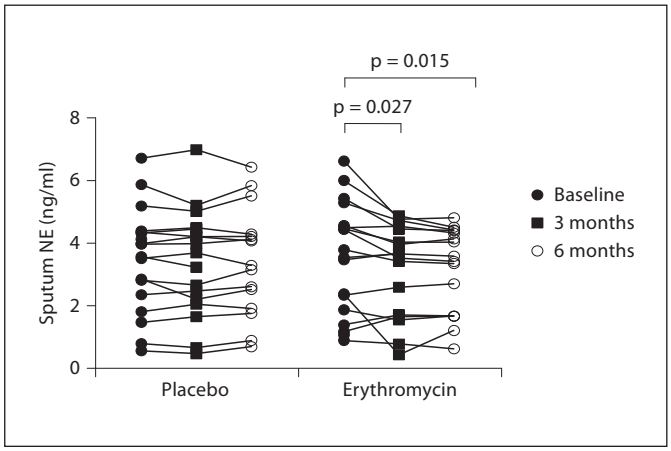

Fig. 2. Effect of erythromycin on induced sputum NE concentration (ng/ml) in COPD patients at baseline and after 3 and 6 months of treatment (p = 0.024, erythromycin vs. placebo after 6 months of treatment).

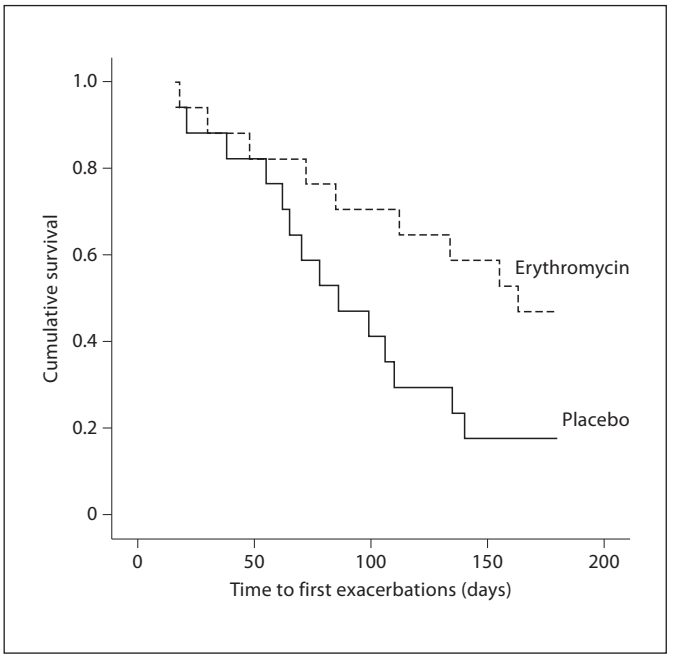

Fig. 3. Kaplan-Meier curves showing the proportion of patients without an exacerbation (cumulative survival analysis) versus time to the first exacerbation for the placebo and erythromycin groups (p = 0.032).

Table 1. Demographic and baseline characteristics

Parameters	Placebo	Erythromycin
Patients, n	18	18
Age, years	69.3 ± 7.1	68.8 ± 8.1
Males, n (%)	16 (88.9)	15 (83.3)
FEV_1, liters	1.02 ± 0.41	1.12 ± 0.47
FEV_1, % of predicted	42.1 ± 18.6	44.3 ± 17.9
FVC, liters	2.48 ± 0.8	2.37 ± 0.7
FEV_1/FVC, %	48.6 ± 8.4	46.9 ± 8.0
Smoking, pack-years	41.1 ± 19.8	42.3 ± 17.9
Body mass index	23.2 ± 3.1	23.4 ± 3.6
Present treatment, n (%)		
Inhaled corticosteroid	8 (44.4)	7 (38.8)
Theophylline	11 (61.1)	10 (55.6)
Inhaled anticholinergic	9 (50)	10 (55.6)
Inhaled β-adrenergic	13 (72.2)	14 (77.8)

Means ± SD and numbers (%) are shown. No significant difference was found between the placebo and macrolide groups.

lymphocytes and macrophages did neither significantly change during treatment nor between the two groups. Erythromycin treatment significantly reduced NE concentrations in sputum supernatant compared with baseline (p = 0.027 after 3 months and p = 0.015 after 6 months). This reduction was also significantly greater compared with 6-month placebo treatment (p = 0.024; fig. 2).

Sputum Bacteriology

At baseline, 16 stable COPD patients (placebo = 7/erythromycin = 9) had bacterial growth in the sputum, of which 4 patients had more than one organism. The main three bacterial pathogens in the sputum were *Streptococcus pneumoniae* (placebo = 3/erythromycin = 3), *Haemophilus influenzae* (placebo = 3/erythromycin = 2) and *Branhamella catarrhalis* (placebo = 2/erythromycin = 2). At the 6-month time point, 14 sputum samples from the patients had significant bacterial growth and 3 of these specimens had growth of more than one organism. The main three pathogens that were detected were *S. pneumoniae* (placebo = 3/erythromycin = 2), *H. influenzae* (placebo = 2/erythromycin = 2) and *B. catarrhalis* (placebo = 2/erythromycin = 1). There was no difference in the detection rate regarding the three main microorganisms between the two groups at baseline or after 6 months of treatment.

Health-Related Quality of Life

SF-36 and SGRQ scores were not significantly different at baseline between the placebo and erythromycin groups. In the erythromycin group, general health scores in the SF-36 had significantly improved after 3 (p = 0.04)

Table 2. Effects of erythromycin on inflammatory cells (10^6/ml) in induced sputum

Parameters	Placebo			Erythromycin			p value vs. placebo
	baseline	3 months	6 months	baseline	3 months	6 months	
Total cells	3.76 (3.26–4.26)	3.73 (3.24–4.20)	3.69 (3.22–4.15)	3.74 (3.24–4.24)	3.19 (2.84–3.53)*	3.20 (2.81–3.59)*	0.021
Neutrophils	2.81 (2.44–3.19)	2.79 (2.44–3.13)	2.75 (2.44–3.07)	2.80 (2.40–3.20)	2.29 (1.87–2.70)*	2.25 (1.90–2.60)*	0.005
Macrophages	0.71 (0.60–0.82)	0.75 (0.64–0.86)	0.72 (0.60–0.83)	0.70 (0.58–0.82)	0.66 (0.54–0.78)	0.69 (0.50–0.89)	0.296
Lymphocytes	0.22 (0.17–0.27)	0.21 (0.15–0.26)	0.20 (0.14–0.26)	0.20 (0.17–0.23)	0.19 (0.16–0.21)	0.19 (0.15–0.23)	0.946

Means and 95% confidence intervals are shown. * $p < 0.001$ vs. baseline in the erythromycin group.

Table 3. Effects of erythromycin on SF-36 and SGRQ scores in COPD

Parameters	Placebo			Erythromycin		
	baseline	3 months	6 months	baseline	3 months	6 months
SF-36						
General health	40.0 (30.7–49.3)	38.3 (29.8–46.8)	39.8 (30.6–48.9)	37.2 (28.4–46.1)	40.1 (29.5–50.8)*	45.1 (34.7–55.5)*
Physical functioning	59.7 (48.8–70.7)	59.2 (49.0–69.3)	57.6 (47.3–67.9)	61.1 (51.3–70.9)	64.0 (53.7–74.3)	65.0 (55.2–74.8)
Bodily pain	80.7 (71.3–90.1)	79.9 (71.7–88.2)	80.4 (71.2–89.6)	79.9 (70.8–89.0)	77.2 (69.0–85.5)	80.4 (71.1–89.7)
Vitality	68.1 (61.3–74.8)	64.1 (57.0–71.2)	64.1 (56.6–71.6)	68.1 (60.4–75.7)	69.0 (61.9–76.1)	66.1 (56.0–76.2)
Role physical	45.8 (21.6–70.1)	41.0 (18.2–63.8)	46.2 (21.6–70.8)	44.4 (20.5–68.4)	48.2 (27.9–68.6)	45.1 (21.3–68.8)
Role emotional	59.3 (48.6–69.9)	59.6 (49.4–69.9)	60.0 (48.6–71.4)	59.3 (41.7–76.8)	63.6 (44.4–82.7)	63.4 (45.4–81.5)
Social functioning	60.2 (48.3–72.1)	59.1 (47.9–70.3)	58.8 (45.9–71.7)	62.7 (51.7–73.7)	70.7 (58.2–83.2)*	70.5 (57.6–83.4)*
Mental health	76.0 (65.9–86.1)	75.0 (66.5–85.6)	76.5 (67.2–85.8)	75.3 (65.4–85.2)	80.7 (73.8–87.6)	80.0 (70.7–89.3)
SGRQ						
Total score	50.3 (43.1–57.5)	50.4 (42.9–57.9)	50.7 (42.9–58.6)	49.7 (41.8–57.6)	48.1 (39.0–57.1)	47.7 (38.4–56.9)
Symptom	66.3 (62.0–70.5)	64.0 (58.1–69.8)	66.3 (60.4–72.2)	67.5 (59.1–76.0)	64.5 (55.1–73.9)	64.0 (55.2–72.9)
Activity	56.1 (53.5–58.7)	55.0 (52.4–57.6)	55.6 (51.7–59.4)	57.3 (51.9–62.7)	54.9 (46.5–63.3)	53.5 (45.8–61.1)
Impact	36.3 (34.5–38.2)	35.0 (33.3–36.7)	36.5 (33.2–39.8)	35.6 (25.7–45.4)	37.6 (27.8–47.4)	38.4 (28.5–48.3)

Means and 95% confidence intervals are shown. * $p < 0.05$ vs. baseline.

and 6 months ($p = 0.02$) compared with baseline (table 3). Similarly, there was also a significant reduction in social functioning scores after 3 and 6 months compared with baseline ($p = 0.045$ and $p = 0.044$, respectively). However, these changes were not significantly different between the erythromycin and placebo groups.

For the other SF-36 scores including physical functioning, pain index, vitality, physical role limitation, emotional role limitation and mental health, there were no significant changes in either group (table 3). There were also no significant changes in SGRQ scores in either group (table 3).

Exacerbations

There were a total of 31 moderate-severe exacerbations over the 6-month treatment time, of which 20 occurred in the placebo group and 11 in the erythromycin group. The proportion of patients with at least one exacerbation was 78% in the placebo group and 50% in the erythromycin group. Patients who received erythromycin treatment experienced a mean of 0.61 exacerbations (95% confidence interval: 0.42–0.80) per patient/6-month follow-up, compared with 1.11 (0.83–1.39) exacerbations in the placebo group ($p = 0.042$), with a relative risk of an exacerbation in the erythromycin group of 0.554 compared with the placebo group (table 4). Exacerbations occurring during the study were more frequent in patients with lower FEV_1% of predicted. Sex, age and smoking index did not affect exacerbation frequency.

The median time to the first exacerbation was 86 days in the placebo group and 155 days in the erythromycin group. Kaplan-Meier survival analysis showed that eryth-

Table 4. Analysis of exacerbation frequency and exacerbation duration in 36 COPD patients with Poisson distribution

Parameters	Relative risk	95% CI		p value
		lower	upper	
Erythromycin	0.554	0.314	0.979	0.042
Male sex	0.475	0.205	1.101	0.082
	Regression coefficient			
Age	0.036	–0.015	0.088	0.166
FEV_1: % at baseline	–0.049	–0.081	–0.017	0.005
Smoking: pack-years	–0.007	–0.022	0.008	0.993

CI = confidence interval. Relative risk and regression coefficients (B) (generalized linear model) are shown for binary variables and continuous variables with 95% confidence intervals, respectively.

romycin significantly delayed the time to the first COPD exacerbation compared with placebo (p = 0.032; log-rank test; fig. 3).

Safety

Five patients dropped out of the study. In the erythromycin group, 2 patients discontinued their participation in the study, 1 patient because of abdominal pain after erythromycin treatment and the other had a complication of left heart failure. In the placebo group, three patients dropped out of the study, 2 patients because of respiratory insufficiency and another due to an unknown cause. In none of the participants was treatment associated with laboratory or ECG abnormalities.

Discussion

This randomized controlled trial in COPD patients showed beneficial effects of erythromycin therapy on airway inflammation, exacerbations and health status. A recent clinical trial reported a decrease in exacerbations in COPD patients treated with erythromycin [30]. Our data not only confirm these results but also show that erythromycin treatment reduced airway inflammation, as shown by a decreased number of neutrophils and neutrophil activity in sputum.

COPD is a chronic inflammatory process, and neutrophils are considered to be a key player in this process. Neutrophils are elevated in the sputum of COPD patients [8]. They are ascribed an active role in the disease process and are associated with an accelerated decline in FEV_1 [6, 7]. Neutrophils contribute to the pathogenesis of COPD by the production of proteases, oxidants and inflammatory mediators. Therefore, interventions that reduce neutrophil recruitment might be anticipated to modify the progression of COPD.

The aim of this study was to investigate whether erythromycin is effective in suppressing airway inflammation and neutrophil activity in COPD patients. Induced sputum is a technique that has been used to investigate cellular and cytokine changes in response to oral and inhaled steroids. The present study showed a reduction in the total cell number in sputum, accounted for by a reduction in neutrophils, after erythromycin treatment. Treatment with erythromycin also significantly reduced NE concentration in sputum supernatant, suggesting diminished airway neutrophil activation following treatment. This may also induce a beneficial effect by reducing proteolytic damage in the airways. Reductions in neutrophil numbers and NE concentrations have been reported following macrolide treatment in patients with other chronic airway diseases, such as DPB, bronchiectasis [24] and asthma [17]. One mechanism by which erythromycin may reduce neutrophil numbers is via improved phagocytosis of apoptotic cells. Alveolar macrophages treated with macrolide antibiotics show increased phagocytic capacity for apoptotic neutrophils [31] and epithelial cells [32].

The SGRQ and the SF-36 are the most commonly used to assess health status in clinical trials on COPD patients. However, there was no significant improvement in SGRQ scores and most domains of SF-36 following erythromycin treatment except for social functioning scores and general health scores compared with placebo.

We found the exacerbation rate was significantly decreased in patients treated with erythromycin compared with the placebo group. The relative risk of a moderate-severe exacerbation was reduced by 35% in the erythromycin group compared with the placebo group. The mechanism by which macrolides affect COPD exacerbation frequency is still unknown. In vitro studies of macrolides have shown a reduction in cellular inflammatory responses at the nuclear level [25, 26].

Other studies have shown similar results with macrolides regarding the reduction of exacerbations [30, 33] and decreased inflammatory cytokines in sputum [34]. However, there are also some conflicting results showing a negative effect of macrolides on health status, sputum inflammatory cytokines and exacerbations [35, 36]. The

reason for these conflicting results is unknown, but may be due to differences in the study design, including the use of clarithromycin rather that erythromycin in these studies [35, 36]. In our study and most previous studies with positive results [30, 33], it has been shown that at least 6 months of macrolide treatment can have beneficial effects, particularly on COPD exacerbations, effects being similar to those observed following long-term macrolide treatment of patients with DPB and cystic fibrosis [14]. The reduction in exacerbations in our study was greater than that shown in the study by Seemungal et al. [30] and less than that shown in the study by Suzuki et al. [33]. Apart from differences in the study design, it is interesting to note that the use of inhaled corticosteroid was different between these two studies, with the study by Seemungal et al. [33] having the most and the study by Suzuki et al. [30] having the least number of patients concomitantly on inhaled corticosteroids, which may have influenced the differences in the magnitude of the reduction in exacerbation frequency between these studies. However, although erythromycin treatment decreased exacerbations of COPD, health status did not improve, which is surprising given the significant effect that exacerbations have in reducing health status.

Our study has some limitations. The sample size was small and a 6-month treatment duration may be insufficient to assess exacerbation rate completely. Larger and longer studies with erythromycin will be required to determine if these positive effects can be replicated.

Previous studies have shown that lower airway bacterial colonization in patients with COPD is related to exacerbation frequency [37–39]. One possible explanation for the improvement in exacerbations with erythromycin may result from its antibacterial effect. However, no significant differences were found in the number of positive sputum cultures for the main COPD pathogens during the 6-month treatment between the two groups. A positive bacterial culture was found in 42% of stable COPD patients before treatment and in 45% after treatment, values of bacterial colonization that are in agreement with those in other studies [37–39].

In summary, we have shown beneficial effects of erythromycin treatment in COPD patients, i.e. reductions in the number of neutrophils and in the NE concentration in the sputum concomitant with a significant reduction in COPD exacerbations. These results will confirm the potential useful benefit of macrolide therapy in the management of COPD patients.

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