


The Effect of Telithromycin on Inflammatory Markers in Chronic Obstructive Pulmonary Diseases

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ABSTRACT

Aim: To evaluate the anti-inflammatory effect of telithromycin on sputum interleukin-8 (IL-8), tumor necrosis factor- α (TNF- α), myeloperoxidase (MPO) levels in patient with chronic obstructive pulmonary diseases (COPD).

Methods: Thirty four patients with mild to moderate COPD were enrolled in this prospective, single center, double-blind, placebo controlled study. Subjects received either telithromycin or placebo for 10 days. Before and after treatment period spirometric tests, arterial blood gas analyses were performed, sputum samples were taken for measurement of sputum inflammatory markers, and sputum was induced.

Results: There was no statistical difference in baseline clinical or laboratory parameters between groups. After the treatment, the induced sputum IL-8, TNF- α , MPO levels is similar compared with pretreatment levels.

Conclusion: In this study, anti-inflammatory effects of telithromycin in stable COPD patients were not demonstrated. Further studies are needed to determine the clinical significance of these findings.

Key Words: Chronic obstructive pulmonary diseases, Telithromycin, Interleukin-8, Myeloperoxidase, Tumor necrosis factor- α .

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterized by sputum production, bacterial colonization, neutrophilic bronchial airway inflammation, poor health status and recurrent infective exacerbations. Macrolide antibiotics have been shown to improve symptoms and exacerbation rates in chronic lung disease (1).

Some studies have demonstrated in patients with asthma that macrolide antibiotics improve bronchial hyperreactivity and reduce steroid-dependent asthma (2,3). The recognition of airway inflammation which is a relatively new finding in COPD is associated with neutrophils, as well as with neutrophilic markers including interleukin-8 (IL-8), tumor necrosis factor- α (TNF- α), myeloperoxidase (MPO), rather than eosinophils (2,3). There are many researches about anti-inflammatory effect of macrolides, however in our knowledge there is no information about anti-inflammatory effect of telithromycin in literature. In this study, our aim is to evaluate anti-inflammatory effect of telithromycin with inflammatory markers such as IL-8, MPO and TNF- α .

MATERIALS and METHODS

Patients aged between 41 and 76 years with COPD diagnosed according to GOLD (Global Initiative for Chronic Obstructive Lung Diseases) criteria were included to study (4). The inclusion criteria included prebronchodilator forced expiratory volume in 1 second (FEV₁) < 80% of predicted normal, prebronchodilator FEV₁/forced vital capacity (FVC) < 70%, irreversible airway obstruction defined as improvement in FEV₁ value < 10% after inhalation of albuterol 200 μ g, smoking history of > 20 pack/years, and no exacerbation or respiratory tract infection within 6 weeks before the study. Patients were excluded if they had history of asthma, clinical signs of right heart failure, were hospitalized or had been admitted to hospital because of exacerbation in last 6 weeks before the study had positive sputum culture, were currently receiving antimicrobial treatment, had suspected or known hypersensitivity to macrolides, or had experienced severe renal insufficiency (requirement of hemodialysis or peritoneal dialysis) or hepatic failure (presence of severe jaundice and/or abnormal liver function test values). The ethic committee approved the study, and all patients provided informed consent.

Study Design

This was a prospective, single-center, double-blind, placebo-controlled study. Physical examination, pulmonary function tests, and routine laboratory evaluations were performed prior to study. Sputum cultures were done in 24 patients who produced spontaneous sputum. Blood drawn for later analysis of inflammatory markers, and sputum was induced by inhalation hypertonic saline solution. An induction process was done in all patients even they produced spontaneous sputum. Thirty four patients who met the inclusion criteria were then randomly divided into 2 groups: 17 participants were given telithromycin 800 mg orally once daily in addition to bronchodilator therapy for 10 days, while the other 17 patients were given a placebo plus a bronchodilator. Outcome measurement including pulmonary function tests and blood was drawn, and sputum induction was repeated at the end of 10 days. Treatment adherence was encouraged by weekly calls from the coordinator and measured by pills counts. Patients were considered adherent if they took at least 80% of medication.

Sputum Induction

The sputum was induced as described by Pin at al (5). All subjects were pretreated with albuterol 200 μ g, administered by metered dose inhaler with spacer (Aero-chamber). For the induction process, Pulmo-Aide ultrasonic nebulizer with an output of 0.35 mL/min and particle size of 5 μ m was used and 3% hypertonic saline was nebulized. Nebulization time minutes consisted of 5 minutes intervals until a maximum nebulization time of 30 minutes was reached. Peak expiratory flow was measured after each period of inhalation. Subjects were asked for rinse their mouth and swallow the water and blow their nose to minimize combination with saliva postnasal drip. They were then encouraged to cough sputum into a sterile container. The procedure was continued until either a sufficient amount of sputum was obtained (>1 mL) or maximum nebulization time of 30 minutes was reached (6).

Sputum Analysis

The sputum samples were processed within 2 hours according to the validated protocol with modification (7). The volume of induced sputum was determined and mixed with an equal volume of 1% sputalys in

freshly diluted to 1% by addition of distilled water. The mixture was incubated at room temperature for 20 minutes and, during this time, vortexed every 5 minutes to ensure homogenization and maximize cell dispersion. The sputum samples were processed with dithiothriol (DTT) (Sigma-Aldrich Inc., USA.) and Dulbecco's Phosphate Buffered Saline Solution (PBS) (Sigma-Aldrich Inc., USA.) before Enzyme Linked-Immuno-Sorbent Assay (ELISA). To stop the effect of DTT (dithiothreitol) on cell suspension, an equal volume of phosphate-buffered saline added. The mixture was then centrifuged at 1500 rpm for 10 minutes. Supernatants were aspirated and stored at -70°C for later analysis of inflammatory markers. The cell pellets were resuspended with phosphate-buffered saline to obtain a final volume of 2-5 mL, then filtered through a gauze (pore size obtain ~ 1 mm) to remove mucus and cell debris.

Clinical Measurements

Spirometry (FEV₁, FVC and FEV₁/FVC) was performed using a Microlap (Medical International Research, UK; accuracy ±3%; flow range ±16 L·s⁻¹). Reversibility of FEV₁ was assessed by repeating spirometric tests 20 min after inhalation of salbutamol (400 µg), given through a volume spacer by a trained member of the lung function department.

The supernatant recovered from the sputum processing was decanted and stored at -70°C and later analyzed for IL-8, TNF-α and MPO. Assays of inflammatory markers of sputum samples were performed with ELISA method using commercially available kits (IL-8 and TNF-α Biosource International, Inc. USA, MPO

R&D Systems Europe, Abingdon, UK) and automated ELISA device (Biomaster Biokit, Spain). The intra and inter assay coefficient of variation (CV) for these assays have previously been shown to be <10 % (5). In the current study, the lower limits of detection were as follows: IL-8 0.0125 nM; TNF- 0.1 pM; and MPO 1 µM; all values below these limits were regarded as zero. The intra- and inter-assay CVs were: IL-8 <9 %; TNF-α <10 and <15 % respectively.

Statistical Analysis

All statistical analysis were performed using SPSS version 11.5 program. Quantitative variables were expressed as mean ± SD. Grouped variables were expressed as percentage (%). Before and after treatment changes in inflammatory markers, pulmonary function tests, and were determined by nonparametric Wilcoxon signed rank test value. Comparison between the placebo and the treatment groups were performed with a p value <0.05 was considered statistically significant.

RESULTS

Totally 34 participants, mean age 60, 12±10.01 years, were included to the study. There was no statistically significant difference in either baseline clinical or laboratory parameters between the groups. No clinically significant changes in physical examination or clinical laboratory data were observed during the study. The clinical characteristics of the study population were shown in (Table 1).

The patients had no positive sputum cultures to indicate airway infection. Both volume and the appear-

Table 1. Characteristics of study population.

Parameter	Telithromycin	Placebo
Patients	17	17
Mean age	60.2±10.1	57.0±10.7
Smoking period	36.2+/-13	20.2±21.2
FEV ₁ % pre-treatment	50.8±0.55	53.81±20.61
FEV ₁ % post-treatment	64.85±20.4	56.20±22.34
FVC % pre-treatment	57.9 ± 19.2	53.81±20.61
FVC % post-treatment	69.5 ± 17.8	63.80±21.47

FEV₁: Forced expiratory volume in 1 second, FVC: Forced vital capacity.

ance of sputum were similar between the groups. Serum and sputum IL-8, TNF- α and MPO levels were not statistically significant both group (Table 2).

Five adverse effects were reported during the study: The most frequent adverse effects were gastrointestinal intolerance; vaginitis was seen in one case. All patients were completely adherent to therapy and no subject was drawn for the adverse effect.

Pulmonary function tests were also performed; we observed significant changes between pre-treatment FEV₁ and post-treatment FEV₁ (54.9 \pm 17.8 vs 73.5 \pm 14.6 p<0.01), Pre-treatment, post-treatment PEF value are also significant in telithromycin group (40.7 \pm 12.2 vs. 60 \pm 15.6 p<0.01). On the other hand no significant change was present in placebo group. According to the improvement of pulmonary function test in telithromycin group, we can propose that telithromycin may have an anti-inflammatory effect. However we didn't observed same improvements in sputum analysis.

DISCUSSION

Chronic obstructive pulmonary disease (COPD) is characterized by progressive airflow limitation associated with chronic inflammation. IL-8, TNF- α and MPO are known important inflammatory factors in airway inflammation. Suppression of airway inflammation might have positive effect in COPD (4).

Macrolides are used for therapy of bacterial infections and as immunosuppressive agents. In recent years, a variety of reports have been published demonstrating anti-inflammatory effects of macrolide anti-bacterial agents. For instance, in carrageenin-induced pleurisy in the rat, roxithromycin, clarithromycin and eryth-

romycin exerted anti-inflammatory activity which was thought to depend on their ability to prevent the production of inflammatory mediators and cytokines (8).

MacLeod et al. after 14 days clarithromycin treatment in patients with chronic sinusitis, reported reductions inflammatory markers and improvements clinical symptoms (9). However, Banerjee et al, was determined no significant effect of clarithromycin on sputum neutrophil number and cytokine levels, with 3 months placebo controlled study that evaluated the effects of clarithromycin on sputum total cells, neutrophils, IL-8, TNF- α , LTB4 and neutrophil elastase levels (1).

Telithromycin, the first member of the ketolide antibacterials which was derived from Erythromycin-A, has good activity against community-acquired respiratory pathogens, including multiple-drug-resistant strains of *Streptococcus pneumonia* (10).

To our knowledge, this is the first study to evaluate the anti-inflammatory effect of telithromycin in Stable COPD with 10 days treatment. We investigated whether telithromycin was effective suppression of airway inflammation in COPD. We used induced sputum for evaluating for airway inflammation.

In conclusion, this randomized-controlled trial showed that oral telithromycin had no anti-inflammatory effect in stable COPD patients. However, we observed improvements of pulmonary functions in telithromycin group. Further studies that include more patients are necessary to clarify possible anti-inflammatory effect of telithromycin.

Table 2. Comparison of induced sputum cells and inflammatory marker levels.

Parameter	Telithromycin treatment			Placebo		
	Before	After	p value	Before	After	P value
IL-8	0.87 \pm 0.82	0.95 \pm 0.67	0.20	0.68 \pm 0.77	0.87 \pm 0.85	0.48
MPO	0.65 \pm 0.55	0.76 \pm 0.63	0.48	0.60 \pm 0.53	0.61 \pm 0.40	0.42
TNF- α	0.13 \pm 0.98	0.39 \pm 0.62	0.86	0.28 \pm 0.40	0.24 \pm 0.38	0.61

IL-8: Interleukin 8, MPO: Myeloperoxidase, TNF- α : Tumor necrosis factor- α .

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