Available online at www.sciencedirect.com
ScienceDirect
Pulmonary Pharmacology & Therapeutics 20 (2007) 46–51
PULMONARY PHARMACOLOGY & THERAPEUTICS
www.elsevier.com/locate/ypupt

Long-term treatment with theophylline reduces neutrophils, interleukin-8 and tumor necrosis factor-α in the sputum of patients with chronic obstructive pulmonary disease

Hirotoshi Iiboshi[a], Jun-ichi Ashitani[a,*], Shigeki Katoh[b], Arisa Sano[a], Nobuhiro Matsumoto[a], Hiroshi Mukae[c], Masamitsu Nakazato[a]

[a]*The Third Department of Internal Medicine, Miyazaki Medical College, Miyazaki University School of Medicine, Kihara 5200, Miyazaki 889-1692, Japan*
[b]*The Department of Cell Regulation, Kagawa University School of Medicine, Kagawa, Japan*
[c]*The Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan*

Received 26 July 2005; received in revised form 27 October 2005; accepted 15 November 2005

Abstract

Background: The non-selective phosphodiesterase inhibitor theophylline has bronchodilator/anti-inflammatory properties and is widely used in the treatment of airways diseases. We determined the effect of long-term theophylline treatment on airway inflammation in patients with chronic obstructive pulmonary disease (COPD).
Populations and methods: Seventeen stable COPD patients were enrolled in the 12-month study. Theophylline was administered at 400 mg/day. We studied changes in symptoms, spirometry, sputum volume, and sputum inflammatory cytokines levels. We also examined the effects of theophylline on the release of inflammatory cytokines in vitro by measuring interleukin (IL)-8 and tumor necrosis factor (TNF)-α levels from lipopolysaccharide (LPS)-stimulated neutrophils and THP-1 cells.
Results: Forced vital capacity was increased and sputum IL-8 levels decreased after 4 weeks of theophylline treatment. After 6 months of theophylline treatment, forced expiratory volume in 1 s was increased, and neutrophils counts and TNF-α levels in sputum were reduced. Levels of IL-8 and TNF-α released by LPS-stimulated THP-1 cells were reduced by treatment with theophylline at 10 μg/ml. In contrast, IL-8 levels released by LPS-stimulated neutrophils were reduced by treatment with theophylline at 100 μg/ml.
Conclusion: Our clinical study of small population showed that long-term treatment with theophylline seems to reduce airway inflammation in stable COPD patients.

Keywords: Theophylline; COPD; IL-8; TNF-α; THP-1; LPS; Neutrophils

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a common disease characterized by progressive airflow limitation that is closely related with chronic airway inflammation. Improvement of airflow limitation is the main target of COPD treatment, although airway inflammation is difficult to control because of the poor response to glucocorticosteroids. International guidelines recommend the use of theophylline and β2-adrenergic receptor agonists over glucocorticosteroids in the treatment of COPD [1]. The unresponsiveness to glucocorticosteroids in COPD is thought to be caused by decreased histone deacetylase (HDAC) activity [2,3]. HDAC enzyme regulates inflammatory gene expression and plays a role in the suppression of inflammatory genes by glucocorticosteroids [4]. It has been reported that oxidative stress reduces HDAC activity in alveolar macrophages of COPD patients compared with healthy smokers and normal volunteers [3]. Theophylline induces significant increases in HDAC levels

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV, forced expiratory volume; FVC, forced vital capacity; HDAC, histone deacetylase; IL, interleukin; LPS, lipopolysaccharide; TNF, tumor necrosis factor

*Corresponding author. Tel.: +81 985 85 2965; fax: +81 985 85 8305.
E-mail address: jashi2@fc.miyazaki-u.ac.jp (J.-i. Ashitani).

1094-5539/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pupt.2005.11.008

[3], and may therefore restore steroid responsiveness in COPD patients [5].

Theophylline is widely used as a bronchodilator in patients with airflow limitation diseases such as bronchial asthma and COPD [6]. The efficacy of theophylline was clinically verified and demonstrated to rely largely on the mechanism of bronchodilation, although recent studies also showed some anti-inflammatory effects [7,8]. Studies on the effect of short-term theophylline treatment on airway inflammation and comparisons between theophylline and β2-adrenergic receptor agonists on bronchodilation concluded that theophylline was not an effective bronchodilator in COPD patients [9,10]. Airway inflammation in COPD has been studied by examination of sputum [11]. Previous studies showed elevated levels of interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) in the sputum of COPD patients [12,13]. A clinical study of small population revealed that short-term treatment with theophylline did not reduce inflammatory cytokine levels in the sputum of COPD patients [9]. However, to our knowledge, long-term treatment of COPD patients with theophylline has not been evaluated by analysis of inflammatory cytokines in the sputum. To determine whether theophylline acts as an anti-inflammatory agent in COPD patients, we investigated the effects of long-term theophylline treatment on symptoms, spirometry, and inflammatory cytokines in the sputum in COPD patients.

2. Methods

2.1. Study setting and population

Patients with stable COPD (mean age, 71.6 $\pm$ 1.2 years, $\pm$SD) and no previous treatment with theophylline, β2-adrenergic receptor agonists, or glucocorticosteroids were recruited for this study. The diagnosis of COPD was established using the following criteria: (1) history of cigarette smoking, with a minimum of 20 pack-years, in patients who all were current smokers at time of evaluation; (2) no exposure (occupational or otherwise) to other substances known to cause lung disorders; (3) absence of atopy, i.e., negative skin tests for common allergen extracts and no personal or family history of allergic disease; (4) no history of systemic or other pulmonary disease or congenital and/or acquired systemic immunodeficiency; (5) forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) $<$70% and FEV1 after inhalation of 200 μg salbutamol $<$80% predicted; and (6) no exacerbation within the preceding month. Informed consent was obtained from all patients at study entry.

2.2. Study protocol

Before study entry, patients were screened by clinical history, physical examination and chest radiograph. A symptom score was recorded for each COPD patient as described: three symptoms such as sputum quantity, cough and dyspnea were graded for severity using a 4-grade system (grade 1 = 0; grade 2 = 1; grade 3 = 2; grade 4 = 3) [14]. For accurate assessment, the scorings were made using the following criteria; sputum quantity per day was defined as grade 1; $<$2 ml, grade 2; 2–10 ml, grade 3; 10–50 ml, and grade 4; $>$50 ml. Cough score was defined according to times per day as grade 1; none, grade 2; 1–9, grade 3; 10–29, and grade 4; $>$30. Dyspnea score was defined as grade 1; shortness of breath when hurrying on the level or walking up a slight hill, grade 2; walks slower than people of the same age on the level or stops for breath while walking at own pace on the level, grade 3; stops for breath after walking 100 m, and grade 4; stops for breath while talking or wearing clothes. The scores were also recorded in prescribed forms every day and the total marks for 1 week prior to visit were defined as the symptom score of each complaint.

After 2 weeks of course observation under medication-free conditions, sputum sampling and pulmonary function tests were performed. Patients were given 400 mg/day of an oral sustained-release theophylline (Purdue Pharma L.P., Pickering, Canada) once a day for 12 months. Symptom scoring, sputum sampling and spirometry were performed and plasma theophylline concentrations were measured in all patients at 4 weeks, 6 months, and 12 months after initiation of treatment. When theophylline concentration in plasma reached above 15 μg/ml or adverse effects of theophylline such as nausea and tachycardia appeared, administration of theophylline was withheld at once and the patient was withdrawn from the study. The upper limit of theophylline concentration in plasma for dropout was set based on safety in previous study for the elderly in COPD [15].

2.3. Sputum processing

The first sputum produced after waking in the morning was collected and the volume was assessed by weight in "ml". The sputum sample was diluted with 0.1% dithiothreitol, mixed for 30 min, filtered and then centrifuged at 300*g* for 10 min. The supernatant was decanted, aliquoted and stored at –40 °C for cytokine assays. After slides were made with a cytospin, two slides were fixed in acetone/methanol (1:1) and stained with May–Grunwald–Giemsa for differential cell counts of leukocytes and squamous cells. A sample was considered adequate when the percentage of squamous cells was lower than 20% [16,17]. To correct for the variable salivary contamination, the results of differential leukocyte counts were expressed as percentages of nucleated cells excluding squamous cells, which was performed by an observer blinded to the subjects' clinical characteristics.

2.4. Cell culture and stimulation

THP-1 cells, an undifferentiated human pro-monocytic cell line, were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and cultured in RPMI 1640 medium (Life Technologies, Rockville, MD)