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Airway inflammation and tiotropium treatment in stable COPD patients

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Background/aim: Chronic obstructive pulmonary disease (COPD) is an inflammatory disease of the lung associated with progressive airflow limitation. The aim of this study is to assess the influence of tiotropium treatment on airway inflammation and symptoms in stable COPD patients.

Materials and methods: Inflammatory markers were measured in the expired breath condensate fluid (EBC) before starting tiotropium treatment and at the end of the first month.

Results: Twenty-two patients (81% men) with a mean age of 65.4 ± 10.1 years completed the study. The mean nitrotyrosine and 8-isoprostane levels for oxidative stress markers in EBC before and after treatment were 4.5 ± 2.3, 3.5 ± 1.9 pg/mL (P = 0.06) and 7.3 ± 10.8, 8.1 ± 11.7 pg/mL (P = 0.28), respectively. The mean interleukin-6 and tumor necrosis factor-alpha levels for inflammation markers in EBC before and after treatment were 1.03 ± 1.1, 0.77 ± 0.8 pg/mL (P = 0.41) and 27.8 ± 2.6, 29.2 ± 5.7 pg/mL (P = 0.36) respectively. The mean symptom scores decreased significantly with tiotropium and a mean increase of 124.6 ± 0.86 mL was observed in a lung function test (FEV1).

Conclusion: Although a 4-week treatment with tiotropium did not modify any of the inflammatory or oxidative stress markers in EBC fluid, tiotropium treatment helps to control symptoms in COPD.

Key words: Tiotropium, inflammation, airways, COPD

1. Introduction

Chronic obstructive pulmonary disease (COPD), which is an important health problem and an increasing cause of mortality throughout the world, is characterized by a slowly progressive and irreversible reduction in the maximum expiratory flow due to a persistent abnormal inflammatory process in the pulmonary tissue (1). As a result of inhaled toxic gases, mainly tobacco smoke, increased oxidative stress with reactive oxygen species (ROS) and increased inflammation with neutrophils, macrophages, and CD8+ T lymphocytes form a vicious cycle that is implicated in the remodeling of the airways and lung parenchyma (2,3). Examination of exhaled breath condensate (EBC) is a noninvasive method for studying the composition of airway lining fluid. EBC is mainly formed by water vapor but also contains cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha-alpha (TNF-α) which have potent neutrophil chemotactic and activation properties (4,5), and also 8-isoprostane, which mediates certain aspects of oxidative injury (6). Thus, EBC measurements are useful for monitoring and assessing the efficacy of pharmacological therapy with various inflammatory and oxidative stress biomarkers in the respiratory tract (7–10).

The overall aims for COPD treatment are to prevent further decline in lung functions, treat exacerbations, and control symptoms like cough and dyspnea (11). Guidelines suggest starting a long-acting bronchodilator therapy when patients with COPD are symptomatic and once-daily long-acting anticholinergic bronchodilator tiotropium bromide is approved as a maintenance therapy. Since parasympathetic cholinergic pathways arising from the vagus nerve are implicated in the pathophysiology of airflow limitation in COPD, inhaled tiotropium bromide is very effective in reversing vagally mediated bronchoconstriction and decreasing hyperinflation, by blocking muscarinic receptors located in the airway smooth muscle in patients with COPD (12–15). Acetylcholine provides the dominant innervations of the airways and it has been shown that acetylcholine receptors activate the release of chemotactic factors that trigger granulocyte migration (16). Therefore, it can be suggested that

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antagonizing the muscarinic receptors can modulate the inflammatory reactions. Although the role of tiotropium in the management of COPD is well documented, its effect on airway inflammation in stable COPD is not clear. A number of studies have been performed examining these effects of tiotropium upon various measures of inflammation with conflicting results (17,18). The aim of this study was to see if airway inflammation and oxidative stress markers in EBC would be changed by a 4-week course of tiotropium therapy, given at a dose of 18 μg daily.

2. Materials and methods

2.1. Patients

Thirty patients with stable COPD were recruited from the outpatient department of our university hospital during a 2-month summer period. All patients were at least 40 years of age, with a smoking history of at least 20 pack-years, and they had all ceased smoking at least 6 months prior to enrollment. Inclusion criteria were: 1) postbronchodilator forced expiratory volume in first second (FEV_1)/forced vital capacity (FVC) ratio of <70% and FEV_1 of ≥50% of the predicted value, 2) reversibility with inhaled beta-2 agonists of less than 12% of the predicted FEV_1, 3) stable COPD defined as no acute exacerbation within the preceding 3 months, 4) no history of systemic disease or other pulmonary disease, 5) no therapy with inhaled or systemic corticosteroids within 3 months prior to entry into the study, and 6) no history of asthma, rhinitis, or atopy. Exacerbations were defined as an increase in or the new onset of more than 1 respiratory symptom (cough, sputum, sputum purulence, wheezing, or dyspnea) lasting 3 days or more and requiring treatment with an antibiotic or a systemic corticosteroid.

Informed content was obtained from the patients and this study was approved by the ethics committee of our university.

2.2. Spirometric tests and symptom scores

Spirometric tests, EBC, and symptom scores were ascertained upon entry into the study and after 4 weeks of treatment. All patients were assessed within 2 weeks for treatment compliance and side effects. Pulmonary function tests were performed by the standard method using a dry rolling-seal spirometer. Three technically adequate maneuvers were required and the best values for FVC and FEV_1 were accepted.

Each subject was evaluated for symptoms in detail. Dyspnea was scored as "0" for no symptoms, "1" for 1–2 episodes of breathlessness daily, "2" for 3 or more episodes, and "3" for breathlessness most of the time. Cough was scored as "0" for none, "1" for 1–2 episodes daily, "2" for 3 or more episodes, or "3" for persistent cough. Sputum production was ranked as "0" for none, "1" for production only on rising, "2" for occasional sputum production, and "3" for frequent episodes.

2.3. Exhaled breath condensate and markers

EBC was collected over 10–15 min of quiet breathing using a condenser EcoScreen (Jaeger, Germany) according to standard protocol and using a nasal clip. After rinsing their mouths, the recruited subjects breathed tidally through a mouthpiece that was connected through a unique one-way valve to a cooled collection tube where vapors, aerosols, and moisture in the breath condensed along the walls of the tube. The design of the system prevented salivary contamination of EBC. Each subject was asked to breathe through the device, while wearing a nose clip, for 10 min so that more than 1 mL of EBC could be collected from each subject. EBC was transported to the analytical laboratory in tightly closed and cooled containers and stored at –70 °C until analysis/further examination. EBC samples were collected in the morning, from 0900 to 1000 hours. Concentrations of IL-6, TNF-α, 8–isoprostane, and nitrotyrosine in EBC were determined by a 2-site sandwich quantitative enzyme-linked immunosorbent assay (ELISA) using commercially available kits (Chemikline). The markers' concentrations were expressed in pg/mL. The concentration of all markers in the samples was calculated by comparison to the curve obtained with different concentrations of standards included in each kit. Tests were done twice for validation.

2.4. Statistical analysis

Parametric data were expressed as the mean ± SEM and were compared using Student's t-test. Comparisons between baseline and end of treatment data from the treatment and control groups were made using Wilcoxon's signed rank test (2-tailed). Correlations between different parameters were tested with Spearman's rank test. In each case, $P < 0.05$ was considered significant.

3. Results

Initially, a total of 30 ambulatory patients with a medical history of clinical and radiological findings consistent with stable COPD were included in the study. During the follow-up period, 3 patients were excluded from the study for failure to take the medication consistently and 3 patients did not come to their last visit. One patient had an upper airway infection during this period and 1 patient had an appendectomy. Therefore, the results presented are an analysis of 22 subjects (81% men), with a mean age of 65.4 ± 10.1 years (Table 1). All of the patients continued on their preexisting therapies with 18 μg of tiotropium added once daily. The 1-month course of treatment with inhaled tiotropium was well tolerated, with no significant side effects except for minor oral dryness in 4 patients. All patients underwent EBC procedures without any complications.

After the treatment period, none of the markers changed significantly in EBC. A 4-week treatment with

Table 1. Demographic data of all patients.

	n
Number (male/female)	22 (18/4)
Age (years)	65.4 ± 10.1
Smoking history pack-years	45.58 ± 6.3
Spirometry	
FVC (mL)	2505 ± 151.7
FEV_1 (mL)	1568 ± 150.48
Degree of COPD	
Mild	6
Moderate	16

FVC: Forced vital capacity, FEV_1 forced expiratory volume in first second, COPD: chronic obstructive pulmonary disease.

tiotropium did not modify any of the inflammatory or oxidative markers evaluated. The mean nitrotyrosine and 8-isoprostane levels for oxidative stress markers in EBC before and after treatment were 4.5 ± 2.3, 3.5 ± 1.9 pg/mL (P = 0.06) and 7.3 ± 10.8, 8.1 ± 11.7 pg/mL (P = 0.28), respectively. The mean IL-6 and TNF-α levels for inflammation markers in EBC before and after treatment were 1.03 ± 1.1, 0.77 ± 0.8 pg/mL (P = 0.41) and 27.8 ± 2.6, 29.2 ± 5.7 pg/mL (P = 0.36), respectively (Table 2).

The mean symptom scores decreased significantly with tiotropium. Analysis of symptoms showed that there were significant changes in cough, dyspnea, and sputum scores between the baseline and after 1 month of tiotropium treatment (Table 3). A mean increase of 124.6 ± 0.86 mL was observed in FEV_1.

4. Discussion

Our results suggest that treatment with inhaled tiotropium in stable COPD patients for a period of 4 weeks has a positive effect on symptom scores, but this therapy failed to improve inflammation and oxidative stress in EBC as assessed by levels of IL-6, TNF-α, 8-isoprostane, and nitrotyrosine.

Acetylcholine is the primary parasympathetic neurotransmitter in the airways and activates the postsynaptic muscarinic receptors present on airway smooth muscle and submucosal glands (19). Increased cholinergic activity in COPD induces inflammation and mucus secretion and contributes to airflow obstruction, airway wall edema, and airway remodeling (20). Persistent inflammation and oxidative stress are cornerstones in the pathogenesis of COPD. Peta et al. (17) showed that tiotropium decreased the lipopolysaccharide (LPS)-induced increase in neutrophils, goblet cells, collagen deposition, and muscularized microvessels in a guinea pig model of COPD. Similarly, Ohta et al. (21) found that tiotropium reduced airway inflammation and Th2 cytokine production in bronchoalveolar lavage fluid (BALF) in both an acute and chronic murine model of ovalbumin-induced asthma. The goblet cell metaplasia, thickness of airway smooth muscle, and airway fibrosis were all significantly decreased in tiotropium bromide-treated mice. In another animal study, Wollin et al. (22) investigated the antiinflammatory activity of tiotropium on cigarette smoke-induced pulmonary inflammation in mice. One hour before smoke exposure, animals were treated with tiotropium by inhalation (0.01–0.3 mg/mL) for 5 min; 18 h after the last cigarette smoke exposure BALF collection was performed. Concentration-dependent tiotropium inhibited pulmonary neutrophilic inflammation with an half maximal inhibitory concentration (IC_{50}) of 0.058 mg/mL and a maximum inhibition of 60% at 0.3 mg/mL, and release of leukotriene B (4), interleukin-6, and TNF-α were dose-dependently reduced. In our study, we failed to demonstrate any antiinflammatory or antioxidant effect of tiotropium. This could be because all the above-mentioned studies were animal studies. Acetylcholine acts via 2 classes of receptors: muscarinic and nicotinic. It was also shown that various lung cells express different patterns of receptors. For example, fibroblasts expressed predominantly the M2 type and alveolar macrophages expressed predominantly the M3 type of receptors (23). Moreover, the endogenous use of nonneuronal acetylcholine is likely to vary with tissue type and pathology (24). Thus, pharmacodynamic (subtype receptor specificity) and pharmacokinetic (dose, distribution, half-life time) properties might be different for human and animal lung cells.

Table 2. Influence of treatment on markers in exhaled breath condensate.

	Before treatment	After treatment	P
Nitrotyrosine (pg/mL)	4.5 ± 2.3	3.5 ± 1.9	0.06
8-isoprostane (pg/mL)	7.3 ± 10.8	8.1 ± 11.7	0.28
IL-6 (pg/mL)	1.03 ± 1.1	0.77 ± 0.8	0.41
TNF-α (pg/mL)	27.8 ± 2.6	29.2 ± 5.7	0.36

IL-6: interleukin 6, TNF-α: tumor necrosis factor alpha.

Table 3. Influence of treatment on symptom scores.

	Before treatment	After treatment	P
Mean symptom score	2.3 ± 0.66	1.51 ± 0.59	<0.001
Cough	2.36 ± 1.04	2.04 ± 0.73	<0.001
Dyspnea	2.59 ± 0.85	1.4 ± 0.59	0.004
Sputum production	2.04 ± 0.84	1.59 ± 0.66	<0.001

One of the clinical trials that compared the antiinflammatory effects of salmeterol/fluticasone, tiotropium/fluticasone, and tiotropium alone in induced sputum showed that all treatment groups failed to reduce the numbers of total cells, neutrophils, and macrophages in induced sputum. Only 12 weeks of treatment with salmeterol/fluticasone caused a significant reduction in IL-8 and matrix metallopeptidase-9 compared with tiotropium alone. Thus, in this clinical trial, the authors also failed to show any antiinflammatory effect of tiotropium (18). Similarly, Powrie et al. (25) also found that there were no differences between the start and the end of a 1-year course of tiotropium in sputum, serum IL-6, and myeloperoxidase levels in 48 COPD patients in vivo. Surprisingly, they found that tiotropium therapy was associated with a significant increase in the concentration of sputum IL-8. They explained this by suggesting that sputum cytokine measurements might not be an optimal means of assessing airway inflammation. Although in our study we used EBC for assessing the effect of tiotropium, our results were similar with induced sputum.

Isoprostanes are a new class of lipids, isomers of the conventional enzymatically derived prostaglandins, which are produced in vivo primarily by a free radical-catalyzed peroxidation of polyunsaturated fatty acids (26,27). Increased concentrations of 8-isoprostane, hydrogen peroxide, nitrite, and 3-nitrotyrosine are found in EBC in inflammatory lung diseases. Vacca et al. (28) investigated the ability of tiotropium bromide to inhibit alveolar macrophage (AM)-mediated chemotaxis of neutrophils and release of ROS in 71 COPD patients. They showed that blocking muscarinic cholinergic receptors with tiotropium bromide decreases TNF-α mediated chemotactic properties and ROS release of human AM but tiotropium bromide did not affect cellular IL-8, IL-6, LTB4, GM-CSF, or MIPalpha/SZ release in this study. Supernatant from AM stimulated with LPS-induced neutrophilic migration is reduced by tiotropium in a dose-dependent manner. In our study, a 4-week tiotropium therapy did not significantly decrease nitrotyrosine and 8-isoprostane levels in EBC. Thus, as tiotropium may act in a dose-dependent manner, we thought that higher concentrations of tiotropium might be needed for antiinflammatory and antioxidative effects in vivo. As we showed a positive effect of tiotropium on symptom scores, probably the standard dosage (18 μg daily) is enough for bronchodilation, but for in vivo conditions it may be not enough for an antiinflammatory and antioxidative effect. We also know that bronchial inflammation increases with the severity of the COPD, so including only mild and moderate COPD patients, with less oxidative stress and airway inflammation, may make the demonstration of the antiinflammatory effects of tiotropium more difficult.

Our study has certain limitations. Our patient number is relatively small, but this is because of our strict inclusion criteria. We tried to exclude all comorbid and confounding factors. We collected all our patients during summer in order to prevent seasonal variations. On the other hand, the strength of our study is that both subjective symptoms and objective lung functions with EBC were determined. We also tried to examine both inflammatory and oxidative stress markers.

In conclusion, although in vitro studies suggest that anticholinergics have the potential to control antiinflammatory processes in the lung, our study supports the previous clinical trials showing no improvement in the inflammatory markers in stable COPD patients using long-term inhaled anticholinergics in vivo. We think that further studies are needed to elucidate the different results between in vivo and in vitro studies for the antiinflammatory effects of tiotropium.

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