

Exhaled Interleukine-6 and 8-isoprostane in chronic obstructive pulmonary disease: effect of carbocysteine lysine salt monohydrate (SCMC-Lys)

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Abstract

Chronic obstructive pulmonary disease (COPD) is characterized by an airways inflammation and by an enhanced generation of reactive oxygen species. The aim of our study was to assess the inflammation and the oxidative stress in airways of COPD patients with acute exacerbation of disease and in stability. Furthermore, we investigated the anti-inflammatory and antioxidant effects of 6 months treatment with carbocysteine lysine salt monohydrate (SCMC-Lys) in COPD. We studied 30 mild acute COPD, 10 mild stable COPD and 15 healthy subjects. 8-isoprostane and Interleukine-6 were measured in their breath condensate through immunoassay. Significantly higher concentrations of exhaled 8-isoprostane and Interleukine-6 were found in acute COPD patients compared to stable COPD and healthy controls (21.8 ± 5.1 vs. 13.2 ± 2.0 vs. 4.7 ± 1.8 pg/ml and 7.4 ± 0.9 vs. 5.8 ± 0.2 vs. 2.7 ± 0.6 pg/ml, $p < 0.0001$). COPD patients treated with SCMC-Lys showed a marked reduction of exhaled 8-isoprostane and Interleukine-6 (8.9 ± 1.5 and 4.6 ± 0.8 pg/ml, $p < 0.0001$). These findings suggest that there is an increase of 8-isoprostane and Interleukine-6 concentrations in the breath condensate of COPD patients compared to healthy controls especially during acute exacerbations of the disease. Moreover, we showed an anti-inflammatory and antioxidant effect of short-term administration of SCMC-Lys in COPD, suggesting the importance of a further placebo-controlled study that should evaluate the effects of this drug.

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1. Introduction

Chronic obstructive pulmonary disease (COPD) is predicted to become the third most common cause of death and the fifth most common cause of disability in the world by 2020 (Lopez and Murray, 1998).

The airways inflammation and the increased production of reactive oxygen species in the lung play a key role in the pathogenesis of this widespread respiratory disease (Barnes, 2003; Paredi et al., 2002; MacNee, 2001). Although bronchoepithelial cells contain high levels of antioxidant to compete with such oxidants, in COPD, they are inadequate. Therefore, it is realized an oxidant-mediated epithelial injury that finish to damage the epithelial surface of airways by peroxidizing lipids, disrupting proteins and deoxyribonucleic acid (DNA; Brandolini et al., 2003; Kasielski and Nowak, 2001; Pinamonti et al., 1996).

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Recent studies have shown an increase in inflammatory and oxidative stress markers such as Interleukin-6, Leukotriene B4 (LTB4), 8-isoprostane, hydrogen peroxide (H₂O₂) and ethane in breath condensate of COPD subjects (Biernacki et al., 2003; Carpagnano et al., 2003a,b; Dekhuijzen et al., 1996; Montuschi et al., 2000; Paredi et al., 2000). This new method to collect sample from airways, the breath condensate, has recently aroused interest in research and clinic for its simplicity, practicability, non-invasiveness and especially for the great acceptance by patients (Kharitonov and Barnes, 2001a,b).

The inflammation and the oxidative stress further raise during periods of acute exacerbations of COPD that are known to represent the most common cause of hospitalization and of health care costs (Biernacki et al., 2003; Fletcher and Peto, 1977; Kanner et al., 2001). Also, the mucus hypersecretion, hallmark of COPD, seems to get worse during the exacerbations (Schreiber et al., 2002).

Notwithstanding the key role of mucous in aggravate airflow obstruction, the value of mucolytic therapy is unrecognized at a point that the European Respiratory Society and the American Thoracic Society COPD guidelines discouraged the use of mucolytic in treatment of patients with stable and/or acute COPD (American Thoracic Society Standards, 1995 guidelines; British Thoracic Society guidelines, 1997; Schreiber et al., 2002). Of late, the point of view of researchers and clinicians at this regard seems starting to change inasmuch as some thiol-containing mucolytic drugs (sodium 2-mercaptoethane sulphonate and *N*-acetylcysteine) showed associated with their therapeutical effect, anti-inflammatory and antioxidant capabilities (Gressier et al., 1994; Kasielski and Nowak, 2001).

Only recently also, the thioether group contained in carbocysteine lysine salt monohydrate (SCMC-Lys) has been found to have some capacities to reduce airways inflammation (Barnes, 2003; Pfeifer et al., 1997).

The purpose of the present work was to assess the inflammation and the oxidative stress in airways of COPD patients during an acute exacerbation of diseases and in stability. Furthermore, we studied the anti-inflammatory and antioxidant effects of SCMC-Lys in COPD patients measuring an inflammatory (Interleukine-6) and an oxidative stress marker (8-isoprostane) in their breath condensate before and after 6 months of SCMC-Lys administration.

2. Methods

2.1. Study population

We studied 40 ex-smoker patients with mild COPD (cigarette consumption 32±5 pack/years) and 15 non-smoker healthy subjects with no history of lung disease

(Table 1). All subjects were recruited from the Respiratory Disease Institute, University of Bari, and written informed consent was obtained from all subjects. The study was approved by the Institutional Ethics Committee. The diagnosis of COPD was based on GOLD Guidelines (Pauwels et al., 2001). All COPD patients were ex-smokers, had stopped smoking for at least 1 year, had no history of allergic diseases, peptic ulcer or intestinal malabsorption, were not suffering from chronic congestive heart failure and did not use domiciliary oxygen (Pa_{O2}>60%). Healthy subjects had never smoked, had no respiratory symptoms and had no respiratory tract infection for ≥3 months prior to the study. Exclusion criteria were a known allergy to carbocysteine lysine salt monohydrate (SCMC-Lys).

Thirty COPD patients were enrolled in this study at the hospital admission for treatment of acute exacerbations. Ten patients with stable COPD, used as a control group, were enrolled during semester check visit. An acute exacerbation was defined as worsening of the previous stable clinical status with presence of at least one of the following: increased sputum purulence and/or volume, increased dyspnea, wheeze or chest tightness (British Thoracic Society guidelines, 1997). At enrollment, patient's medical history and general physical condition were recorded. A spirometry, a chest X-ray, blood gases analysis, laboratory tests and breath condensate collection were also carried out. All these measurement were taken in patients with acute exacerbation of COPD before starting a course of a broad spectrum antibiotic (T0) and then repeated after 2 weeks when in a stable condition [no respiratory symptoms, no signs of infection, stable forced expiratory volume in one second (FEV₁) and spirometric bronchodilator response to 500 µg nebulised salbutamol <15% of the baseline; T1]. At this time, 15 COPD patients enrolled received mucolytic therapy (SCMC-Lys 2.7 g daily administrated orally, supplied by Dompè, L'Aquila, Italy; group A), while the other 15 remained untreated (group B) for 6 months (T2). During this period, only further inhaled

Table 1
Subject characteristics

	Mild acute COPD	Mild stable COPD	Healthy subjects
Number	30	10	15
Age (yr)	51±6	50±4	48±7
Sex (M/F)	19/11	5/5	8/7
FEV ₁ (%predicted)	74.4±2.6	78±3.1	101±18%
FVC (%predicted)	84.2±1.8	87±2.8	119±9%
Pa _{O2} (mm Hg)	76.7±3.5	83±2.3	96.1±2.7
Pa _{CO2} (mm Hg)	39.8±2.1	37±1.2	36.3±2.5

Definition of abbreviations: FEV₁—forced expiratory volume in one second; FVC—forced vital capacity. All data are expressed as means±S.D.

steroids and long-acting B2-agonist were allowed. None of patients included were treated with oral steroids. The patients had taken at least 80% of his medication in order to be considered compliant. Throughout the study, each patient visited the hospital each month for clinical check. All adverse events were recorded, and their relationships to tested medication were assessed. No patient presented a new exacerbation during this period.

After 6 months, COPD patients enrolled in the study were recalled for the end-study visit and the last spirometry, blood gases analysis, laboratory tests and breath condensate collection (T2; Table 2).

2.2. Study design

This study was designed to investigate the inflammation and the oxidative stress in airways of COPD patients with acute exacerbation of disease and in stability and to assess the anti-inflammatory and antioxidant effects of a 6-month treatment with SCMC-Lys.

2.3. Pulmonary function testing

Pulmonary function tests were performed prior to the measurement of exhaled breath condensate (EBC). Forced expiratory volume in one second (FEV₁), vital capacity (FVC) and FEV₁/FVC ratio were measured using a dry spirometer (PK Morgan, Gillingham, UK). The highest value of three maneuvers was expressed as a percentage of the predicted normal value.

2.4. Exhaled breath condensate and assay

Exhaled breath condensate was collected by using an EcoScreen (Jaeger, Wurzburg, Germany). The subjects breathed through a mouthpiece and a two-way nonbreathing valve, which also served as a saliva trap. They were asked to breathe at a normal frequency and tidal volume, wearing a nose clip, for a period of 10 min. If subjects felt saliva in their mouth, they were instructed to swallow it. Condensate, at least 1 ml, was collected as ice at -20°C , transferred to Eppendorf tubes and immediately stored at -70°C . Samples were analysed within 3 months from collection.

To exclude saliva contamination, amylase activity was analysed in exhaled breath condensate (EBC).

A specific enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI) was used to measure 8-isoprostane and Interleukine-6 concentrations in breath condensates. The assay was previously validated directly by gas chromatography/mass spectrometry (Carpagnano et al., 2003a,b; Montuschi et al., 2000). The intra-assay and interassay variability were $\leq 10\%$. The detection limit of the assays was 4 and 1.5 pg/ml, respectively.

2.5. Statistical analysis

Data were expressed as means \pm S.D. A Mann Whitney test to compare groups and a Wilcoxon matched paired test were used, and correlations between variables were performed using Spearman's rank correlation test. Significance was defined as a *p* value of <0.05 . The reproducibility of assays was assessed in a separate experiment through the Bland and Altman method, and the coefficient of variation was determined (Bland and Altman, 1996).

Table 2
Acute COPD subjects at T0, T1 and T2

	COPD with acute exacerbation (T0) <i>n</i> =30	COPD after 2 weeks of antibiotic treatment (T1) <i>n</i> =30	COPD after mucolytic therapy (T2) <i>n</i> =15
FEV ₁ (%predicted)	62.5 \pm 2.9	74.4 \pm 2.6*	75.1 \pm 3.2 [#]
FVC (%predicted)	77.1 \pm 1.6	84.2 \pm 1.8*	87.6 \pm 2.2 [#]
PaO ₂ (mm Hg)	59.1 \pm 2.2	76.7 \pm 3.5**	75.7 \pm 2.4 [#]
PaCO ₂ (mm Hg)	44.6 \pm 1.9	39.8 \pm 2.1*	40.3 \pm 1.8 [#]
Buccal temperature ($^{\circ}\text{C}$)	38.3 \pm 0.7	36.1 \pm 0.6*	36.9 \pm 1.1 [#]
Blood neutrophil count ($\times 10^9/\text{l}$)	14.2 \pm 3.0	6.1 \pm 1.4**	6.8 \pm 1.6 ^{###}
CRP (mg/dl)	7.9 \pm 2.9	1.2 \pm 1.1**	0.8 \pm 0.9 ^{###}

Definition of abbreviations: FEV₁—forced expiratory volume in one second; FVC—forced vital capacity; CRP—C-reactive protein. All data are expressed as means \pm S.D.

* *p* < 0.05.

** *p* < 0.01 (T0 vs. T1).

[#] *p* < 0.05.

^{###} *p* < 0.01 (T0 vs. T2).

3. Results

All patients tolerated the SCMC-Lys without any complaints of side effects. The lung function parameters (FEV₁, FVC), the blood gases and the plasmatic neutrophils and PCR showed an improvement in COPD patients hospitalized for acute exacerbation after 2 weeks of antibiotics but did not indicate any significant differences after treatment with SCMC-Lys (Table 2).

No amylase activity was detected in any exhaled breath condensate sample.

3.1. 8-isoprostane

Exhaled 8-isoprostane levels were markedly high in subjects admitted in the hospital for acute exacerbations of COPD (T0; 21.8 \pm 5.1 pg/ml) compared to stable COPD (13.2 \pm 2.0, *p* < 0.001) and healthy controls (4.7 \pm 1.8 pg/ml, *p* < 0.0001; Fig. 1A). Exhaled 8-isoprostane values fell significantly in acute COPD after 2 weeks of treatment with antibiotic treatment (T1 vs. T0; 21.8 \pm 5.1 vs. 14.3 \pm 5.4 pg/

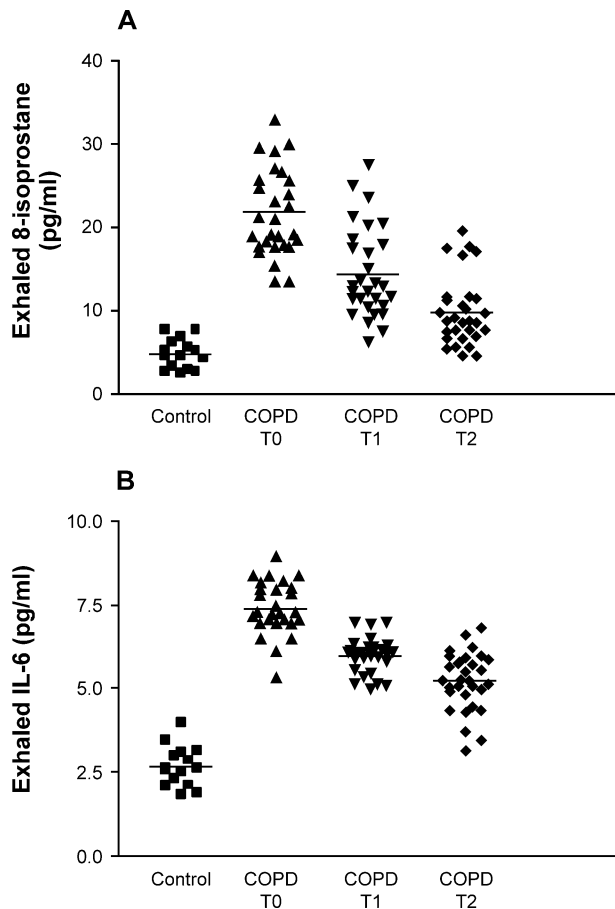


Fig. 1. 8-isoprostane (A) and interleukin-6 (B) concentrations in exhaled breath condensate of healthy and acute COPD and stable COPD subjects. Mean values are indicated by horizontal bars.

ml, $p < 0.0001$). A further reduction of exhaled 8-isoprostane concentrations was observed in 15 COPD subjects treated for 6 months with SCMC-Lys (group A; 8.9 ± 1.5 vs. 13.2 ± 6.2 pg/ml, $p < 0.0001$; Fig. 2A). However, the 8-isoprostane levels in exhaled breath condensate of remaining 15 COPD patients who did not received any therapy (group B) did not change significantly over 6 months (12.7 ± 3.8 vs. 15.4 ± 4.5 pg/ml; T2; Fig. 3A). Concentrations of exhaled 8-isoprostane were significantly lower in group A than in group B at T2 (8.9 ± 1.5 vs. 12.7 ± 3.8 , $p < 0.005$).

No correlation was found between exhaled 8-isoprostane concentration and FEV_1 or FVC.

Reproducibility of exhaled 8-isoprostane measurements was assessed in 10 nonsmoking normal and 10 COPD subjects. In the majority of measurements, the differences between the two 8-isoprostane values lay within ± 2 S.D. [$-0.14(-0.32)$ and $-0.08(1.09)$ pg/ml]. The coefficients of variation for 8-isoprostane were 4.4% and 3.7%.

3.2. Interleukin-6

Exhaled Interleukin-6 levels were markedly high in subjects admitted in the hospital for acute exacerbations of

COPD (T0; 7.4 ± 0.9 pg/ml) compared to stable COPD (5.8 ± 0.2 pg/ml, $p < 0.001$) and healthy controls (2.7 ± 0.6 pg/ml, $p < 0.0001$; Fig. 1B).

Exhaled Interleukin-6 values fell significantly in acute COPD after 2 weeks of treatment with antibiotic (T1 vs. T0; 7.4 ± 0.9 vs. 6.0 ± 0.5 pg/ml, $p < 0.0001$).

A further reduction of exhaled Interleukin-6 concentrations was observed in 15 COPD subjects treated for 6 months with SCMC-Lys (group A; 6.0 ± 0.4 vs. 4.6 ± 0.8 pg/ml, $p < 0.0001$; Fig. 2B).

However, the Interleukin-6 levels in exhaled breath condensate of the remaining 15 COPD patients who did not received any therapy (group B) did not change significantly over 6 months (5.7 ± 0.5 vs. 5.9 ± 0.6 pg/ml; T3; Fig. 3B). Concentrations of exhaled Interleukin-6 were significantly lower in group A than in group B at T2 (8.9 ± 1.5 vs. 12.7 ± 3.8 , $p < 0.005$).

There was a positive correlation between exhaled Interleukin-6 and exhaled 8-isoprostane ($r = 0.8$, $p < 0.0001$; Fig. 4).

Reproducibility of exhaled Interleukin-6 measurements was assessed in 10 nonsmoking normal and 10 COPD subjects. In the majority of measurements, the differences between the two Interleukin-6 values lie within ± 2 S.D.

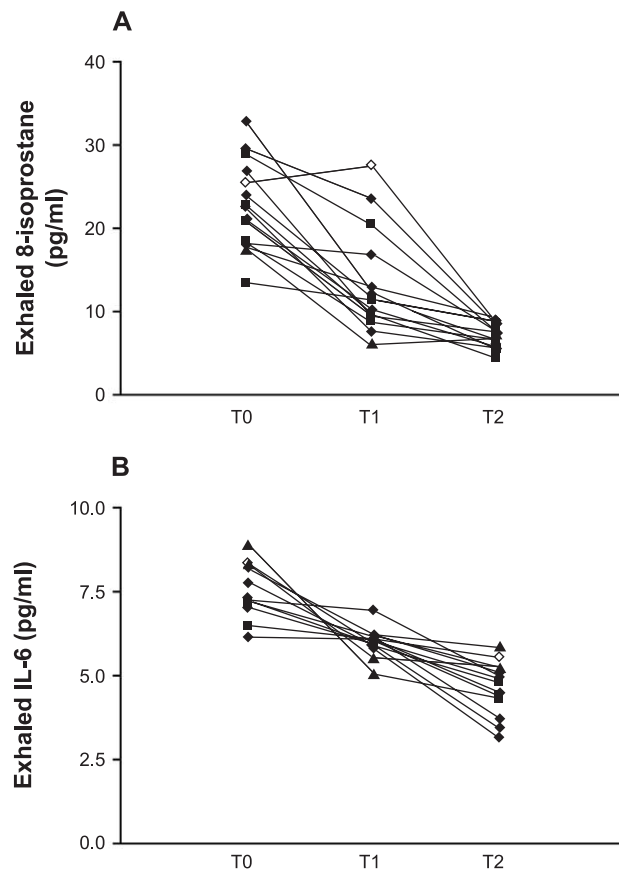


Fig. 2. Exhaled 8-isoprostane (A) and IL-6 (B) concentrations in 15 COPD patients treated for 6 months with SCMC-Lys.

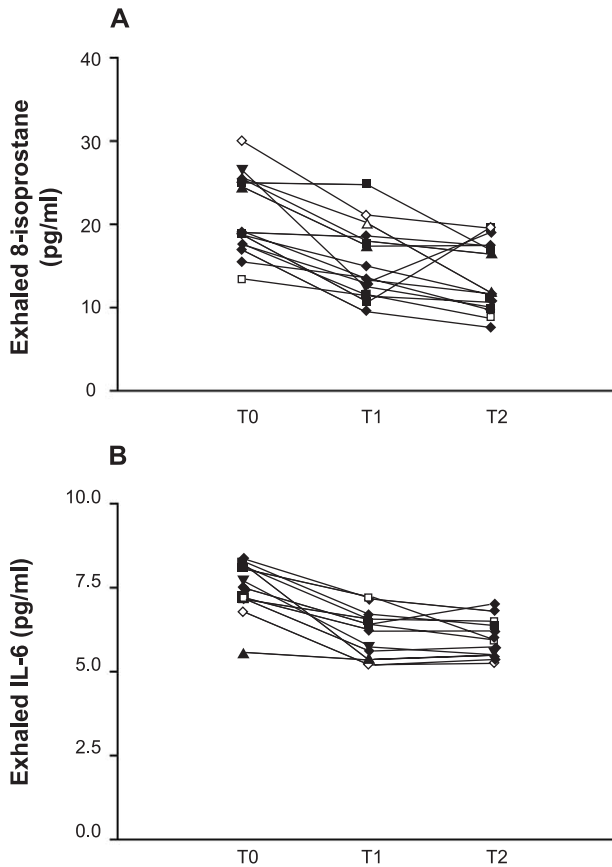


Fig. 3. Exhaled 8-isoprostane (A) and IL-6 (B) concentrations in 15 COPD patients untreated for 6 months.

$[-0.03(-0.24)$ and $-0.04(0.89)$ pg/ml]. The coefficients of variation for Interleukine-6 were 5.9% and 6.4%.

4. Discussion

This study showed significantly higher concentrations of 8-isoprostane and Interleukine-6 in the breath condensate of COPD subjects than in healthy controls, especially during an acute exacerbation of the disease. Both these markers presented a marked reduction after 6 months of daily administration of carbocysteine lysine salt monohydrate (SCMC-Lys) in COPD subjects. However, an improvement of lung function after mucolytic drug was not watched. Finally, a positive correlation was observed between exhaled Interleukine-6 and 8-isoprostane concentrations. This is one of the first studies showing longitudinal data on markers in EBC.

The inflammation and the oxidative stress are implicated in the pathogenesis and progression of COPD (Paredi et al., 2002). Elevated concentrations of oxidative stress markers (hydrogen peroxide, 8-isoprostane and ethane, etc.) as well as inflammatory markers (Leukotriene B₄, Interleukin-6, etc.) demonstrated in breath condensate (Biernacki et al., 2003; Carpagnano et al., 2003a,b; Dekhuijzen et al., 1996;

Montuschi et al., 2000; Paredi et al., 2000), blood (Brandolini et al., 2003; Malo et al., 2001) and urine of patients with COPD patients (Partico et al., 1998), support their key role.

However, monitoring inflammation and oxidative stress in COPD is difficult and may not be reflected by changes in blood and urine markers (Paredi et al., 2002). For this reason, in this study, we use a bodily fluid that reflects airways situation, the breath condensate. The recent interest that this lung's sample is generating is justified by its several advantages compared to other distrectual samples (bronchoalveolar lavage, induced sputum and lung biopsy), such as its completely noninvasiveness, simplicity, practicality and tolerability (Kharitonov and Barnes, 2001a,b).

We measured in the breath condensate of COPD patients the 8-isoprostane (8-epi-prostaglandin-F_{2α}), the predominant isoprostane formed in humans used as quantitative index of oxidative stress in vivo and the interleukin-6, a proinflammatory cytokine produced by epithelial cells and macrophages, expressed in response to various inflammatory stimuli (Carpagnano et al., 2003a,b; Montuschi et al., 2000). The increased exhaled concentrations of both markers that we found in COPD compared to healthy controls further confirm the presence of a marked inflammation and oxidative stress in their airways.

We enrolled in this study COPD patients hospitalized for an acute exacerbation of COPD. In agreement with Biernacki et al. (2003), we observed a further increase of inflammatory and oxidative stress markers in the breath condensate of these patients that significantly fall after 2 weeks of treatment with antibiotics (Partico et al., 1998). We believe that the higher exhaled 8-isoprostane concentrations that we found in acute COPD patients may be due to their more severe conditions as evidenced by the necessity of

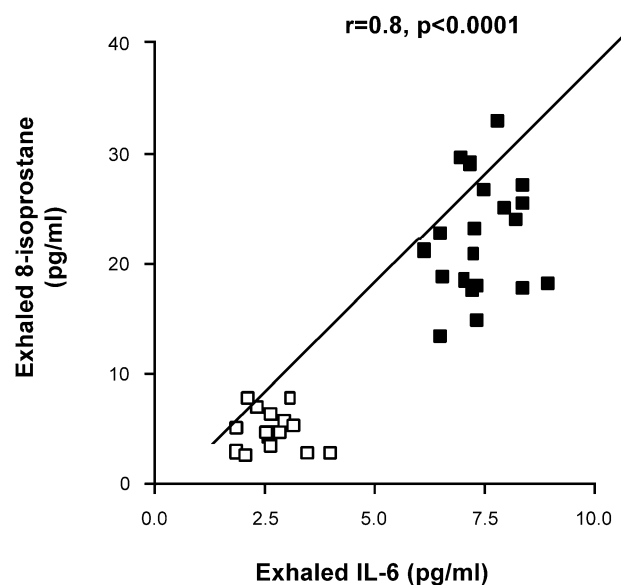


Fig. 4. Correlation between exhaled IL-6 and exhaled 8-isoprostane concentrations in healthy and COPD subjects.

hospital admission compared to those reported by Biernacki who select its patients in a general practice clinic (Biernacki et al., 2003). Our results are also consistent with recent reports that showed a significant augment of other inflammatory markers in the sputum of patients with bacterial exacerbation of COPD that fall rapidly after antibiotic treatment (Bhowmik et al., 2000; Crooks et al., 2000).

The increased burden of oxidative stress further amplifies the inflammatory response. The increased concentrations that we found of exhaled Interleukine-6, which mirror that of exhaled 8-isoprostane, may be mediated through an increase in nuclear factor- κ B, a transcription factor that is activated by oxidative stress and switches on the transcription of inflammatory genes, such as Interleukine-6 (Barnes, 2000). We believe that the correlation observed between exhaled 8-isoprostane and Interleukine-6 levels may support this hypothesis.

This study further wanted to investigate possible effects of carbocysteine lysine salt monohydrate (SCMC-Lys) on inflammation and oxidative stress in airways of humans.

At this aim, 15 COPD subjects underwent 6 months of daily therapy with SCMC-Lys, a some dosage of 2.7 g, used in a previous study in which this drug resulted efficacy and was sure in the prevention of riacutization of COPD (Allegra et al., 1996). SCMC-Lys, whose therapeutic efficacy is commonly related to the ability to replace fucomucins by sialomucins, is often administrated in COPD patients for the positive effects demonstrated in several clinical studies in the treatment of COPD (Allegra et al., 1996; Edwards et al., 1976; Miskovitis et al., 1982).

As a result of the present work, we showed that SCMC-Lys acts by reducing the 8-isoprostane concentrations in breath condensate of healthy and COPD subjects when given continuously for 6 months.

Breath condensate has been previously used in a similar pharmacological study where the effects of a common mucolytic drug, the *N*-acetylcysteine (NAC), were tested on the oxidative stress in subjects with COPD (Brandolini et al., 2003). In this referred study, long-term administration of NAC showed to reduce two oxidative stress markers, the H_2O_2 and the TBARS, in the breath condensate of COPD patients (Brandolini et al., 2003). Although the antioxidant action of NAC and others thiol-containing mucolytic drugs has been largely confirmed by this and other several studies, the capacity of SCMC-Lys as a scavenger of reactive oxygen intermediates has been underestimated until now.

Few evidences in fact appear in literature to confirm of our findings, between those the one of Brandolini et al. (1996) that showed antioxidant properties of SCMC-Lys in cell-free and cellular systems. Only some years later, confirming his previous results, Brandolini et al. observed that SCMC-Lys acts as a selective scavenger of superoxide (HOCL) and hydroxyl radicals (OH°) through oxidation of its thioether group and compared the scavenger capacity of SCMC-Lys to that of GSH (Barnes, 2003).

The antioxidant properties of SCMC-Lys have been also previously confirmed in vitro in three different oxygen radical producing systems represented by BAL from COPD patients, ultrasound treated human serum and cultured human lung endothelial cells challenged with elastase (Pinamonti et al., 1996). In these systems, Pinamonti et al. (1996) also disclosed the antiradical activity of SCMC-Lys towards HOCL and OH and against elastase-induced superoxide production.

In this study, we also showed a reduction of an inflammatory marker, the Interleukine-6 in COPD and healthy subjects, after SCMC-Lys therapy. Ours is not the only finding at this regard. Asti et al. (1995) first supposed that this mucolytic drug can exert an anti-inflammatory action demonstrating that SCMC-Lys acts reducing neutrophil infiltration induced within the airways by intratracheal injection of IL-1 beta. The anti-inflammatory power of SCMC-Lys has also been previously confirmed by Pinamonti et al. (2001) who experimentally showed a decrease in exudates volume and the leukocyte recruitment mediated by SCMC-Lys after pleural inflammation induced by injection of carrageenan.

Although the present study sustains the supposed anti-inflammatory and antioxidant effects of SCMC-Lys, it did not show to improve lung function parameters as well as the other mucolytic drugs (Mesna or NAC; Schreiber et al., 2002). We believe that this finding could explain the lack of correlation that we observed between exhaled Interleukine-6 and 8-isoprostanes and lung function parameters.

However, the spirometric changes associated with exacerbation of COPD are usually very small, so lung function tests are not very useful in detecting exacerbations and are not expected to correlate with inflammatory and oxidative stress markers in airways (Aaran et al., 2001).

In conclusion, our results support the existence of an enhanced inflammation and oxidative stress in airways of COPD subjects that significantly increase during acute exacerbations. They also suggest the usefulness of exhaled markers as the Interleukine-6 and the 8-isoprostane in the clinical management of this widespread respiratory disease.

Furthermore, the reduction of exhaled Interleukine-6 and 8-isoprostane concentrations observed in COPD subjects after short-term administration of SCMC-Lys suggests anti-inflammatory and antioxidant actions of this drug that should be confirmed by a placebo-controlled study. However, further studies are required to elucidate the exact mechanism responsible for the decrease of the inflammation and the oxidative stress mediated by SCMC-Lys.

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