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Original Article

Molecular Mechanisms of Inflammation During Exacerbations of Chronic Obstructive Pulmonary Disease ☆

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ABSTRACT

Introduction: Exacerbations of chronic obstructive pulmonary disease (COPD) are characterised by an inflammatory and systemic response that persists for some time after their clinical resolution. The mechanisms of this inflammatory process are not well known.

Objectives: To explore the inflammatory changes and possible mechanisms during COPD exacerbation.

Methods: We determined the inflammatory cell concentrations in blood and sputum, nitric oxide in exhaled air (FeNO), C-reactive protein (CRP) in plasma, cytokines (IL-6, 8, 1β, 10, 12, TNF-α) and SLPI (leukocyte protease inhibitor) and total antioxidant status (TAS) in blood and sputum, the activity of nuclear kappa B factor (NF-κ B) and of the histone deacetylase enzyme (HDAC) in 17 patients during COPD exacerbation and in stable phase, as well as in 17 smoker and 11 non-smoker controls.

Results: COPD exacerbations are characterised by high levels of FeNO (p<0.05), plasma CRP (p<0.001) and IL-8, IL-1B, IL-10 in sputum (p<0.05) greater activation of NF-κ appaB in sputum macrophages compared with stable COPD and controls. During the stable phase, there continue to be high levels of oxidative stress, SLPI, IL-8, IL-6 and TNF-alfa, with no observed changes in either HDAC activity or in the amount of neutrophils in sputum, despite presenting a significant improvement (p<0.05) in lung function.

Conclusions: Changes were observed in different pulmonary and systemic inflammatory markers during COPD exacerbation, which did not completely resolve during stable phase. However, current treatment does not allow for HDAC activity to be modified, which limits its anti-inflammatory effects..

Mecanismos moleculares de inflamación durante las agudizaciones de la enfermedad pulmonar obstructiva crónica

RESUMEN

Introducción: Las agudizaciones de la enfermedad pulmonar obstructiva crónica (AEPOC) se caracterizan por una respuesta inflamatoria pulmonar y sistémica, que persiste tiempo después de la resolución clínica. Los mecanismos de este proceso inflamatorio no son bien conocidos.

Objetivos: Investigar los cambios inflamatorios y sus mecanismos durante las agudizaciones de la EPOC.

Métodos: Se determinaron las concentraciones de células inflamatorias en sangre y esputo, óxido nítrico en aire exhalado (FeNO), proteína C reactiva (PCR) en plasma, citocinas (interleucinas [IL] 6, 8, 1β, 10, 12, TNF-α) y SLPI (inhibidor de la leucoproteasa), marcadores de estrés oxidativo, la actividad del factor nuclear kappa B (NF-κ appaB) y de la enzima histona deacetilasa (HDAC) a 17 pacientes durante una AEPOC, en fase estable y a 17 controles fumadores y 11 no fumadores.

Resultados: Las AEPOC se caracterizaron por presentar niveles elevados de FeNO (p < 0,05), PCR en plasma (p < 0,001) e IL-8, IL-1β, IL-10 en esputo (p < 0,05) y mayor activación de NF-κ appaB en macrófagos de esputo en comparación con EPOC estable y controles. Durante la fase estable persisten niveles elevados de estrés oxidativo, SLPI, IL-8, IL-6 y TNF-alfa, sin objetivarse cambios en la actividad HDAC ni en la cantidad de neutrófilos en esputo a pesar de presentar una mejoría significativa (p < 0,05) de la función pulmonar.
Conclusiones: Durante las AEPOC se observan cambios en marcadores inflamatorios pulmonares y sistémicos que no se resuelven por completo en fase estable. El tratamiento actual no permite modificar la actividad HDAC lo que limita sus efectos antiinflamatorios.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by the presence of a chronic inflammatory reaction in response to prolonged exposure to tobacco smoke.[1] Patients with COPD frequently present exacerbations, which are a top-ranking social health-care problem because of their negative influence on the quality of life,[2,3] lung function[4,5] and prognosis of these patients[6,7] in addition to social and financial costs.

The pathogeny of COPD exacerbation episodes is not fully understood. There are different studies that demonstrate that these episodes are characterized by an increase in various inflammatory markers, including the number of neutrophils and macrophages in the airway and the concentration of cytokines, particularly IL-6 and IL-8.[8,9] This suggests that the fundamental pathogenic mechanism of COPD exacerbation episodes is a pulmonary "inflammatory flare-up", regardless of the "trigger" that caused it (infection, air pollution, etc.).

The molecular mechanisms of this inflammatory response have also not been fully explained. The activation of nuclear factor kappa B (NF-κ B) plays a central role in the inflammatory response in general and it has been postulated that this is especially true in COPD.[10-12] Under normal conditions, NF-κ B is located in the cell cytoplasm and is made up of two sub-units (p65, p50) that are inactivated by the binding of the I-kB protein. The phosphorylation of the inhibitory sub-unit (I-kB) allows for the translocation to the nucleus of p65 and p50, their binding with specific areas of the genome and the activation of the expression of several pro-inflammatory genes (TNF-α, IL-8, GM-CSF, iNOS, IL-1β).

In the nucleus, DNA is found combined with a group of proteins called histones.[13] The level of acetylation of these histones (measured as histone acetyltransferase [HAT] activity) controls the degree of accessibility of various transcription factors (including NF-κ B) to DNA and, therefore, controls the level of expression of the genes regulated by said transcription factors. The counter-regulatory mechanism of this increased inflammatory expression deacetylates said nuclear histones, activity carried out by the histone deacetylase (HDAC) enzymes. Thus, a greater acetylation of the histones (and therefore greater HAT activity) implies greater NF-κ B activation and a greater transcription of inflammatory genes. Contrarily, the deacetylation of the histones (HDAC activity) implies that the DNA becomes inaccessible to the transcription factors, and there is therefore less inflammatory transcription. Glucocorticoids, powerful anti-inflammatory drugs, inhibit HAT activity mediated by NF-κ B and recruit histone deacetylase (HDAC)[14-16] that revert the histone acetylation process and impede the access of transcription factors (NF-κ B, among others) to the DNA and the transcription of inflammatory proteins. Important pathogenic factors in COPD, such as oxidative stress and tobacco smoke reduce the HDAC activity of the alveolar macrophages[17-19] and promote the persistence of the inflammatory process. It has been suggested that these molecular effects can contribute to explaining the apparent corticoid-resistance observed in patients with stable COPD.[20] The alteration of these mechanisms during COPD exacerbation has not been explored.

Several studies suggest that the resolution of the inflammation can take months after the exacerbation episode.[4] In this study we intend to explore the hypothesis that the inflammatory response after exacerbation is partially attenuated after the inhibition of the inflammatory transcription dependent on NF-κ B although it is not completely resolved due, at least in part, to the persistence of diminished HDAC activity.

The objective of this study was to report the pulmonary and systemic inflammatory changes during COPD exacerbations and to explore the molecular mechanisms related with said changes.

Methods

We recruited patients diagnosed with COPD exacerbation during the first 24 hours after having been admitted by the Emergency Department. These patients had been diagnosed with COPD, defined as a FEV_1/FVC ratio < 70%, determined when stable in accordance with the GOLD criteria, with a history of tobacco use of > 15 packs/year and diagnosis of COPD exacerbation upon admittance. This latter diagnosis was made by an emergency room doctor, who neither participated in nor had knowledge of this study, and who based the diagnosis on the patients' symptoms (increased dyspnea, cough and/or change in expectoration) and complementary tests: arterial gasometry, electrocardiogram, chest radiography and standard blood work-up.

From the respiratory function test lab of the Pulmonology Department, two control groups were recruited, one with a history of smoking more than 15 packs/year and another made up of non-smoker subjects, all with an FEV_1/FVC ratio > 70%.

Excluded from the study were participants with a history of asthma, bronchiectasis, bronchial carcinoma, pneumonia or cardiac failure. Also excluded were those patients who were unable to provide an adequate sputum sample and those who were being treated with theophylline, anti-inflammatory drugs for the treatment of inflammatory diseases such as Crohn's disease or rheumatoid arthritis, or had received treatment with antibiotics or systemic glucocorticoids during the previous 4 weeks. All participants were informed about the nature and purpose of the study and gave their written consent. The study was approved by the Ethics Committee of the Balearic Islands.

Study Design

At the time of admittance, all patients received treatment in accordance with international guidelines, which included nebulized bronchodilators (beta-agonists and anticholinergics), systemic glucocorticoids (oral or intravenous) and antibiotics for all patients that met at least two of the following criteria:

a) increase in the usual dyspnea of the patient
b) fever
c) increase in sputum volume
d) increase in the degree of sputum purulence[21]

If the patient accepted participation in the study, during the first 24 hours after admittance he/she underwent spirometry, arterial

gasometry and FeNO, while sputum and blood samples were taken.

Three months after hospital discharge, an office visit was scheduled in stable phase. The patient was considered to be clinically stable if the patient had had no symptoms of exacerbation (changes in dyspnea, cough and expectoration) nor any need to make changes in his/her usual treatment. If the patient had presented another episode of COPD exacerbation, another office visit was scheduled for three months later. At the visits, all the tests carried out during hospitalization were repeated.

The control subjects, who were recruited in the respiratory function test laboratory and accepted to participate in the study, underwent spirometry, arterial gasometry and FeNO, and induced sputum and blood samples were obtained.

Lung Function

Forced spirometry was carried out with bronchodilator test (GS, Warren E. Collins, Braintree, MA, USA) in all participants following international guidelines both at the time of hospitalization as well as in stable phase.[22] The spirometric reference values used were for Mediterranean populations. If there were previous PFR available, they were used to confirm the diagnosis.

Measurement of Nitric Oxide in Exhaled Air

The measurement of the fraction of exhaled nitric oxide (FeNO) was done with an analyzer (Sievers Instruments Inc. Model 280NOA, Boulder, CO, USA) connected to a Teflon tube, following the recommendations of the *European Respiratory Task Force*, continuing with a methodology previously described in our group.[23]

Sputum Samples and Preparation

The induction of sputum and its processing was done in accordance with a methodology that was standardized in our laboratory and previously reported.[24] Briefly, the sputum obtained was incubated with 0.01 M DTT in *Hanks Buffered SALT Solution* (HBSS), at 4 °C for 15 minutes, diluted with HBSS. It was then filtered through a 50 μm nylon mesh to remove any mucus and detritus without removing cells, and then centrifuged at 790x*g* for 10 minutes. The acellular supernatant was extracted and stored at −70 °C for later determinations. The cell precipitate was resuspended, total cell count was calculated with a hemocytometer by Neubauer, using trypan blue stain for determining cell viability. In order to obtain the cell count, the cellular suspension was cytocentrifuged and stained with Diff-Quick (*International Medical Equipment*, San Marcos, CA 92069, USA). Samples with less than one million cells were considered inadequate and were excluded from the analysis. The cells were incubated on a 6-well plate (*multiwell primaria surface-modified polystyrene*, Falcon 35.3846) at a density of 1 x 10^6 cells/well in 2 ml of medium (RPMI 1640, 10% fetal bovine serum, L-glutamine). After 4 hours, the supernatant was collected and the adhered macrophages were extracted that were later lysated for the extraction of nuclear proteins (*Nuclear extract kit*, Active Motif, Carlsbad, CA 92008, USA).

Determination of Cytokines

The concentration of cytokines (TNF-α, IL-6, and IL-8) was determined in the supernatant by flow cytometry (CBA, *Human inflammation Kit*, BD Biosciences, San Jose, CA, USA) following the instructions of the manufacturer. The sensitivity of the test, according to the manufacturer, was: 3.6 pg/ml for IL-8, 2.5 pg/ml for IL-6, and 3.7 pg/ml for TNF-α. The determination of secretory leukocyte protease inhibitor (SLPI) was done with specific ELISA (R&D Systems, Minneapolis, MN, USA).

Total Antioxidant Status (TAS)

TAS was measured in sputum by means of colorimetric test (Randox Laboratories Ltd, Crumlin, UK) using a methodology that was previously described by our group.[25]

Histone Deacetylase (HDAC) Enzyme Activity

HDAC activity was measured in nuclear extracts of macrophages from the sputum by means of a non-isotopic test using a fluorescent derivative of epsilon-acetyl lysine (HDAC *Fluorescent Activity Assay Kit*, BIOMOL, Plymouth, PA, USA), following the instructions of the manufacturer.

Nuclear Factor Kappa B (NF-κ b) Activity

In nuclear extracts from sputum macrophages, NF-κ B activation was measured using the TransAM NF-κ B p65 Transcription Factor assay kit (*Active Motif, Carlsbad*, CA, USA), following the instructions of the manufacturer.

C-Reactive Protein (PCR)

PCR in plasma was determined by means of the technique of nephelometry, following the standardized technique in the central laboratory of the hospital.

Statistical Analysis

The results are expressed as mean ± SD (or medians and intervals in non-normal distributions). The HDAC activity and NF-κ B, the values of IL-8, IL-6, TNF-α, FeNO, TAS, FEV1, SLPI and PCR presented normal distribution and were compared between the groups with ANOVA. If the ANOVA showed significant differences, the comparisons *a posteriori* or *post hoc* (multiple comparisons) were carried out with the Tukey-Kramer test. For the comparison between the COPD groups during exacerbation and stable phase, the *t* Student's test for paired samples was used. A p value < 0.05 was considered significant. The analysis was completed with Prisma GraphPad software (GraphPad Software Inc., San Diego, CA, USA).

Results

Clinical Data

Seventeen patients with previous COPD diagnosis were recruited during an exacerbation, who were later re-studied in stable phase. The control subjects also studied included 17 smokers and 11 non-smokers with normal lung function. The demographic and functional characteristics of the patients are summarized in table 1. Five patients (29%) with COPD exacerbation had sputum cultures with potentially pathogenic bacteria (PPB) growth: 2 *Pseudomonas aeruginosa*, 2 *Haemophilus influenzae* and 1 *Streptococus pneumoniae* at the time of admittance due to exacerbation and two patients had growth of *Haemophilus influenzae* in stable phase. There were no differences in the parameters analyzed between the patients with positive culture for BPP and the patients with negative culture.

Pulmonary Inflammation Markers

The cell pattern of the sputum shows a predominance of neutrophils in COPD patients during exacerbation, but which also persists during stable phase. Meanwhile, the control subjects show a predominance of macrophages and lymphocytes (table 2).

The exacerbated COPD patients presented higher levels of FeNO that reduced significantly during stable phase (p < 0.05),

Table 1

Demographic and functional characteristics of the participants

	COPD (n = 17)	Smokers (n = 17)	Non-smokers (n = 11)
Age (years)	66.6 ± 1.5	60.8 ± 2.2	64.2 ± 3.1
Sex (M/F)	17/0	14/3	4/7
Packs/year	61.9 ± 3.8	49.8 ± 3.7	0
BMI (kg/m^2)	25.3 ± 1.0	26.03 ± 0.9	27.4 ± 1.6
FEV1 (L)	1.39 ± 0.57	2.92 ± 0.18	2.45 ± 0.16
FEV1 (%)	41.7 ± 15	98.5 ± 2.4	117.5 ± 9.07
FVC (L)	2.79 ± 0.99	3.68 ± 0.9	2.94 ± 0.61
FVC (%)	83.7 ± 8.5	104.1 ± 10.2	112 ± 8.6
FEV$_1$/FVC	49.9 ± 13	79.8 ± 5.8	88.8 ± 4.5
N patients with previous COPD exacerbation	9	0	0
N patients receiving ICS	13	0	0

Values expressed as mean ± standard deviation.

ICS: inhaled corticosteroids; FEV1: forced expiratory volume in one second in stable phase; BMI: body mass index.

Table 2

Sputum cell pattern

	COPD exacerbation	Stable	Smoker	Non-smoker
PMN (%)	47.96 (6.86)	45.93 (8.59)	9.69 (4.5)* †	1.92 (0.71)* †
MCF (%)	14 (9.96)	21.68 (6.46)	72.39 (11.61)* †	75.94 (2.35)* †
LINFOS (%)	38.02 (3.09)	31.93 (5.29)‡	17.9 (7.29)* †	22.07 (3.02)* †

Percentage of cells expressed as mean ± standard deviation.

* $p < 0.001$ compared with COPD exacerbation.
† $p < 0.001$ compared with stable phase.
‡ $p < 0.05$ compared with COPD exacerbation.

observing no differences between the individuals in stable phase and control subjects, in both smokers as well as non-smokers (fig. 1).

During exacerbations, we also found significantly higher levels of inflammatory cytokines IL-8, IL-1β, IL-6 and TNF-α in sputum (fig. 2). IL-8 and IL-1β were significantly lower during stable phase, but IL-6 and TNF-α do not significantly decrease from the exacerbation to the stable phase and remain high compared with control subjects without COPD. The levels of IL-8 in stable phase were significantly higher than in the smoker and non-smoker control subjects. In contrast, the levels f IL-1β reduced to levels similar to that of the controls. The anti-inflammatory cytokines IL-10 and SLPI were higher during exacerbation compared with the controls. The levels of SLPI continued to be high in the stable phase, but the levels of IL-10 lowered in the stable phase to levels similar to those of control subjects (fig. 3).

Systemic Inflammation Markers

The cell pattern in peripheral blood shows a significant increase in polymorphonuclear leukocytes and reduction in lymphocytes in the exacerbated patients compared with stable patients and controls. The patients with stable COPD and smokers also showed a greater number of total leucocytes than the non-smokers (table 3).

During the exacerbations, we found high levels of C-reactive protein (CRP) and fibrinogen in plasma compared with stable phase and with smoker control subjects ($p < 0.001$).

No significant differences were found in the serum levels of IL-6, 8, 1β, 10, 12, TNF-α measured in the different groups.

Inflammation Mechanisms

The determination of indirect indicators of oxidative stress shows a greater oxidative load, translated into a greater presence of antioxidants in the supernatant of the sputum of COPD patients, both stable as well as exacerbated compared with control subjects. Nevertheless, the levels of TAS in sputum were lower during exacerbation than in stable phase ($p < 0.05$) although significantly greater in stable phase than in controls ($p < 0.05$) (fig. 4).

COPD exacerbation was related with greater NF-κ B activity in sputum macrophages that decreased in stable phase ($p < 0.05$) and was greater than in the non-smoker controls ($p < 0.01$) (fig. 4). The HDAC activity in macrophages was lower compared with non-smoker controls ($p < 0.001$), but there was no difference between exacerbation and stable phase. The decrease in HDAC activity correlated significantly with greater levels of IL-8 ($r^2 = 0.38$, $p < 0.05$) (fig. 5). No significant correlations were found between the levels of NF-κ B activation or oxidative stress and the rest of the inflammatory parameters.

Lung Function

The COPD patients presented a significant recuperation of lung function three months after the exacerbation ($p < 0.05$) (fig. 6), finding no correlation with the lung inflammation parameters. There were no significant changes in FVC.
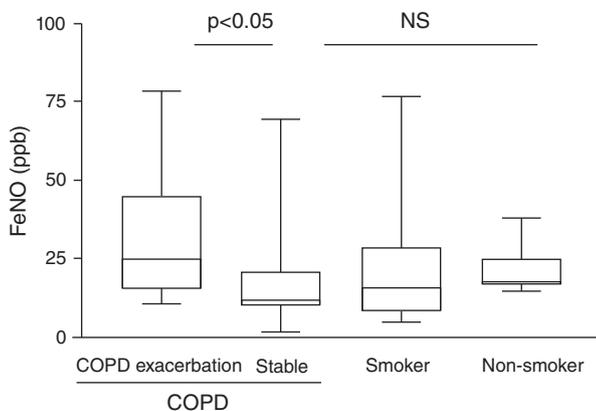

Figure 1. Differences in the exhaled fraction of nitric oxide (FeNO) between patients with COPD exacerbation and stable phase in comparison with smoker and non-smokers controls.
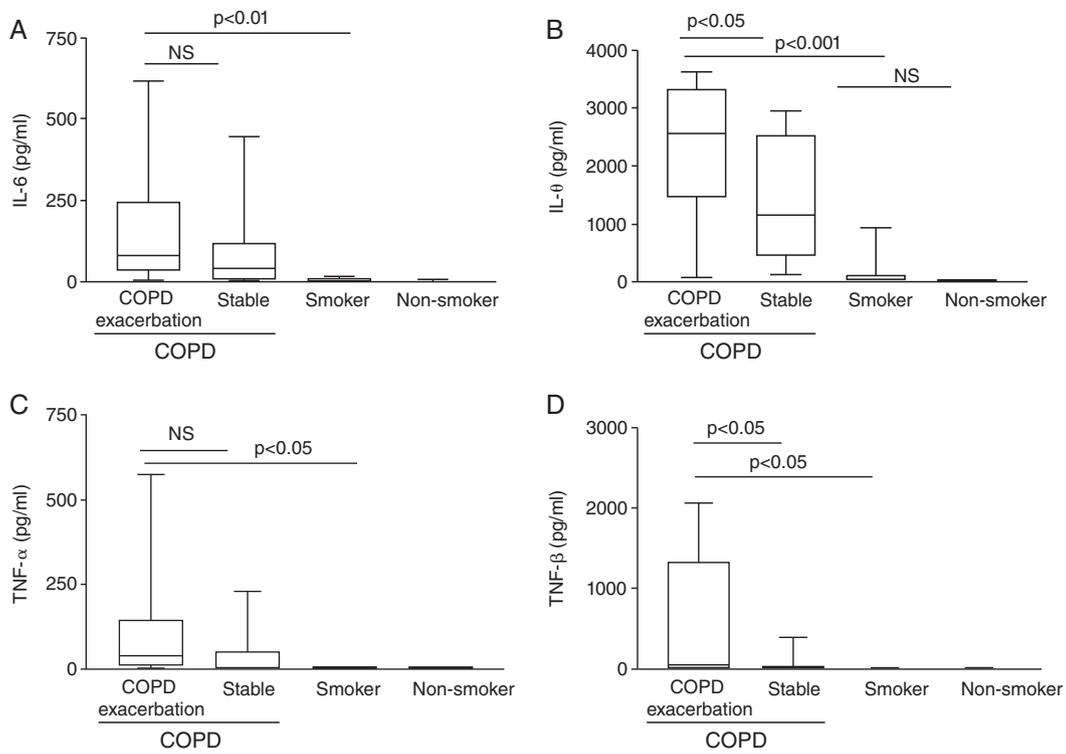
Figure 2. Differences in the sputum concentration of different inflammatory markers: interleukin 6 (IL-6, Panel A), interleukin 8 (IL-8, Panel B), tumor necrosis factor-alpha (TNF-α, Panel C) and interleukin 1 beta (IL-1β, Panel D).

Table 3
Systemic inflammatory markers

	COPD exacerbation	Stable	Smoker	Non-smoker
Leu, total ($\times 10^3/\mu$L)	12.14 (0.97)	8.59 (0.61)‡	7.70 (0.49)‡	6.28 (0.56)* †
PMN, total ($\times 10^3/\mu$L)	10.19 (0.94)	5.54 (0.56)*	4.42 (0.41)*	3.91 (0.68)*
PMN (%)	82 (2.53)	63.68 (3)	56.69 (2.88)	58.94 (4.97)
Lympho, total ($\times 10^3/\mu$L)	1.09 (0.10)	1.95 (0.17)‡	2.36 (0.21)	1.64 (0.15)
Lympho (%)	9.89 (1.30)	23.39 (1.97)‡	31.13 (2.46)	28.57 (3.58)
Eos, total ($\times 10^3/\mu$L)	0.10 (0.04)	0.29 (0.08)	0.26 (0.06)	0.22 (0.08)
Eos (%)	1.28 (0.48)	3.33 (0.93)	3.19 (0.71)	3.81 (1.44)
Fibrinogen (mg/dl)	505.8 (44.14)	471.33 (26.26)	457.50 (31.86)	301.75 (13.53)*
CRP (mg/dl)	9.28 (2.05)	0.79 (0.26)*	1.16 (0.34)*	0.58 (0.13)*

Eos: eosinophils; Lympho: lymphocytes; Leu: leucocytes; PMN: polymorphonuclear leucocytes; CRP: C-reactive protein.
* p < 0.001 compared with COPD exacerbation.
† p < 0.001 compared with stable.
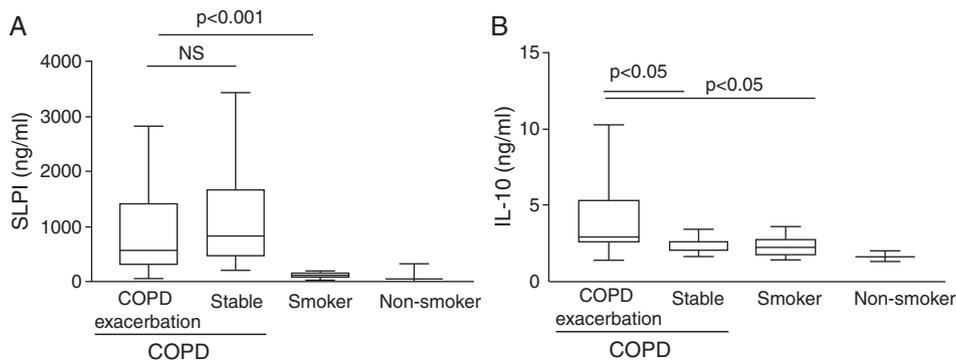‡ p < 0.05 compared with COPD exacerbation.

Figure 3. Concentration in sputum of anti-inflammatory cytokines: serum leukocyte protease inhibitor (SLPI, Panel A) and interleukin 10 (IL-10, Panel B).
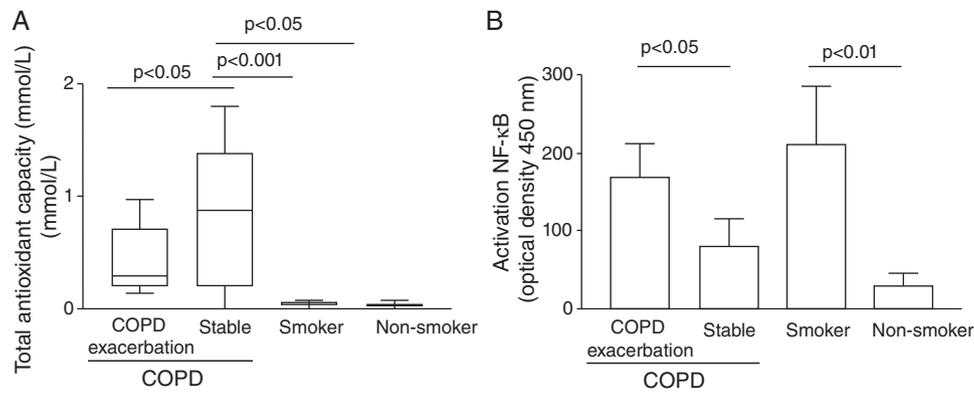
Figure 4. Levels of oxidative stress in sputum measured by total antioxidant capacity (Panel A). Differences in the levels of activation of nuclear factor kappa B (NF-κ B, Panel B) in nuclear extracts of sputum macrophages.
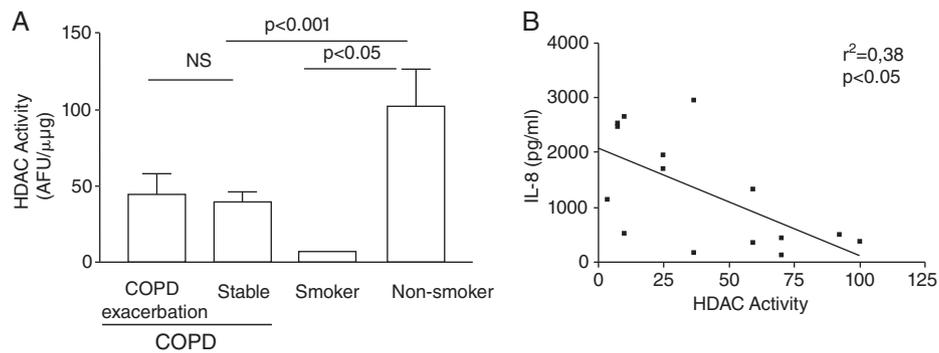

Figure 5. Reduction of HDAC activity in smoker patients and COPD patients (Panel A). This reduction is inversely correlated with interleukin 8 levels in sputum (IL-8, Panel B).

Discussion

In our study, we have found that changes are observed during COPD exacerbation in different pulmonary and systemic inflammatory markers, which are accompanied by a significant deterioration in lung function. This could be related to an increase in oxidative stress and with greater NF-κ B activation. However, several pulmonary inflammation markers, such as the concentration of neutrophils, IL-6 or TNF-α in sputum, do not significantly change from the exacerbation to stable phase, nor does HDAC activity, and its affectation is associated with a greater production of IL-8.

Previous Studies

The molecular mechanisms of the inflammation that occurs during exacerbation have not been thoroughly studied. Some studies have demonstrated a greater activation of nuclear transcription factor NF-κ B (by means of nuclear location with immunocytochemistry, but without studying its real activity)[26] and the role of oxidative stress during exacerbations.[27] This would lead to a greater release of cytokines, inducing greater expression of endothelial adhesion molecules like TNF-α, and chemokines that attract the neutrophils in the airway, like IL-8. Previous studies have already demonstrated an increase in IL-8 and neutrophils during phases of exacerbation.[9] However, no previous study had investigated the molecular mechanism that regulates this inflammatory transcription using the activation of the HDAC activity of the cells implicated in this inflammatory process. This may have special relevance as it has been demonstrated that increasing the HDAC activity during exacerbations, which promotes the anti-inflammatory activity of the glucocorticoids with a stimulator of HDAC activity, such as teofilina in small doses, translates into less pulmonary inflammation and better clinical parameters.[24]

Interpretation of the Results Obtained

Various factors have been identified as triggers for exacerbations: infection, pollution, etc. In addition, there is enough evidence to affirm that inflammation plays a preponderant role in the physiopathology of COPD.[8,28-30] In our study, we have demonstrated
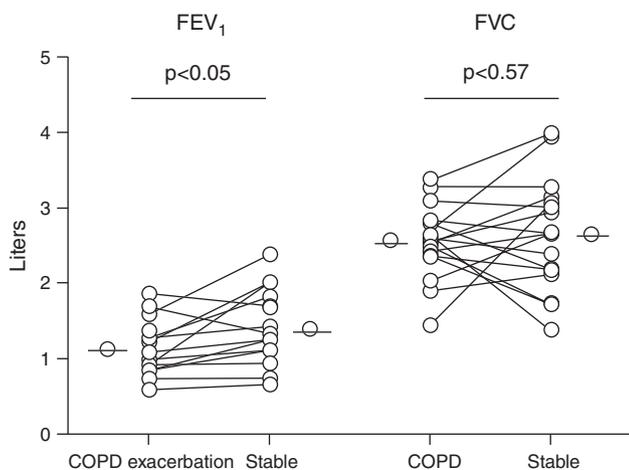

Figure 6. Difference in forced expiratory volume in one second (FEV$_1$) and forced vital capacity (FVC) during exacerbation and during stable phase.

that, regardless of the presence of germs in the sputum of patients with exacerbation, there is an increase in lung and systemic inflammation. Patients with stable COPD have an inflammatory response characterized by an increased presence of macrophages and T CD8+ lymphocytes in the wall of the tracheo-bronquial tree and of neutrophils at the opening of the airways. This cell pattern is modified during exacerbations, where eosinophils and neutrophils predominate.[28] This evidence lets us assume that the inflammation of the airway is amplified; this leads to an increase in bronchial tone, wall edema and increased mucus production, which clinically translates into worsened dyspnea, cough, sputum and alteration in the gas exchange, resulting in the symptoms of exacerbation. The greater amount of inflammation of the airway during exacerbations is also accompanied by more systemic inflammation, which has been suggested to have a role on the higher cardiovascular morbidity.[31] The mechanisms of this inflammatory process are not well known, and we show herein that the oxidative stress and the activation of the NF-κ B factor, where different infectious and other stimuli converge, play a predominating role. However, the molecular mechanism that regulates the transcription of inflammatory genes in the cell nucleus, which is basally reduced in COPD[32] and is responsible for the corticoid-resistance observed in the inflammatory process of stable COPD,[33] does not increase during treatment of the exacerbation with glucocorticoids. We believe that this could be responsible for the persistence of high inflammatory markers three months after the exacerbation. In our study, we found that this activity correlates with the levels of IL-8, a potent chemotactic factor of the neutrophils that may contribute to the persistence of the inflammatory process after the exacerbation, while at the same time translating into a persistence of neutrophils in the airway, as well as persistence of high IL-6 and TNF-alpha levels. Perera et al.[34] relate the inflammatory changes present in COPD exacerbation and the lack of recuperation and recurrence in the following 50 days. They measured the concentrations of IL-6 and PCR in plasma and IL-6 and 8 in the sputum of COPD patients in stable phase, during exacerbation and at 7, 14 and 35 days post-exacerbation. The lack of improvement in the symptoms after the exacerbation was associated with persistent high systemic inflammation. The persistence of neutrophilia in the sputum and oxidative stress in stable phase observed in our study can go along the same lines. Pinto-Plata et al.[35] tried to relate systemic cytokines and the physiological and clinical changes in patients hospitalized due to COPD exacerbation. A significant correlation was found between the changes in IL-6, IL-8 and LTB4 and the changes in FEV$_1$, from 48 hours after admittance until 8 weeks after hospital release.

Clinical Implications

The persistence of the inflammatory process after the exacerbation has a relevant clinical translation. In fact, it is possible that the high rate of re-hospitalizations in the first weeks after exacerbation can be related to the persistence of the inflammatory process.[34] The mechanism responsible can be related with the lack of activation of the HDAC activity. Therefore, drugs directed at promoting HDAC activity would have a relevant clinical translation because, in reducing the neutrophilic inflammatory process that is not sensitive to treatment with corticosteroids, the rate of re-hospitalizations and exacerbations could be reduced in these patients. Obviously, randomized clinical assays are necessary to confirm these proposed clinical implications.

Potential Limitations

The objective of this study was to describe the potential molecular mechanisms that play a role in the underlying inflammatory process in COPD. We have studied sputum microbiology as a possible cause of exacerbation, but the precise role of proinflammatory factors other than bacterial infection, such as environmental pollution, changes in temperature or viral infections, have not been determined. Likewise, the proportion of cultures with PPB is low (29%), and no differences were found with the patients with negative cultures, which can suggest that the molecular mechanism is ultimately the same. The low proportion of patients colonized in stable phase (12%) would not justify the effect observed on either the lack of activation of the HDAC activity or the persistence of elevated parameters.

An important number of patients (76%) were receiving treatment with inhaled corticosteroids, and the effect that this treatment could have had on inflammatory parameters cannot be determined. Another limitation is the low number of non-smoker control subjects analyzed. The sample size does not allow for making associations with clinical parameters but it is sufficient to detect significant changes in several inflammatory parameters.

Conclusions

COPD exacerbations are accompanied by inflammatory changes, at the lung as well as the systemic level, that are not completely resolved after the exacerbation. In the molecular mechanism of the pulmonary inflammatory changes, oxidative stress and nuclear factor NF-κ B participate. The greater activation of nuclear factor NF-κ B observed during exacerbation decreases during stable phase, probably related to glucocorticoid treatment. This is not true for the ultimate mechanism for regulating inflammatory transcription, HDAC activity, which could be responsible for the persistence of neutrophilic inflammation after the exacerbation.

Conflict of Interest

The authors declare having no conflict of interest.

Acknowledgements

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