Lipoxygenase-Derived Arachidonic Acid Metabolites in Chronic Obstructive Pulmonary Disease

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Summary. *Background and Objective. Chronic obstructive pulmonary disease (COPD) is characterized by a persistence of inflammation in large and small airways. We hypothesized that this could be caused by the inability of an inflammatory process to resolve. In the resolution of inflammation, a switching of arachidonic acid metabolism from the production of proinflammatory leukotriene B_4 (LtB_4) to the synthesis of anti-inflammatory lipoxins plays an important role. The aim of our study was to determine the content of lipoxin A_4 (LXA_4) and LtB_4 in induced sputum of patients with exacerbated COPD and to compare it to healthy controls, as well as to analyze the relationship between proinflammatory and anti-inflammatory mediators and an inflammatory cell spectrum in induced sputum.*

Material and Methods. Induced sputum from 17 COPD patients and 7 healthy controls were analyzed for LXA_4 and LtB_4 content and inflammatory cell spectrum.

Results. COPD patients had a significantly lower sputum LXA_4 concentration and LtB_4/LXA_4 ratio compared with healthy controls. A significant negative correlation was found between the LXA_4 concentration and the relative neutrophil count and between the LtB_4/LXA_4 ratio and the relative macrophage count.

Conclusions. COPD patients during the late phase of exacerbation had a suppressed production of LXA_4 and an elevated LtB_4/LXA_4 ratio in induced sputum demonstrating a proinflammatory imbalance. The correction of a balance between proinflammatory and anti-inflammatory eicosanoids by the administration of stable analogues of lipoxins could improve the treatment of chronic obstructive pulmonary disease in the future.

Introduction

Chronic obstructive pulmonary disease (COPD) is a major global cause of morbidity and mortality worldwide. The prevalence of this disease is increasing and is expected to increase in the future (1, 2). It is estimated that COPD causes more than 2.5 million deaths per year worldwide and that by the year 2020, it will rank fifth in the worldwide ranking of the burden of disease (3–5).

COPD is characterized by an airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious agents (3). The nature of abnormality in the course of inflammation is still unknown. We hypothesized that the cause could be the inability of an inflammatory process to resolve. Lipoxins (LXs) play an important role in the resolution of inflammation. They are derivates of arachidonic acid elaborated in an inflammatory area by sequent enzymatic processes realized by 5-lipoxygenase (5-LOX), 12-lipoxygenase (12-LOX), or 15-lipoxygenase (15-LOX). 5-LOX is crucial for the production of lipoxins; however, the major product of this enzyme is leukotriene A_4 (LtA_4) that under the influence of LtA_4 hydrolase transforms to proinflammatory leukotriene B_4 (LtB_4). LtA_4 is taken up by platelets where 12-LOX transforms it to anti-inflammatory LXA_4 and further to lipoxin B_4 (LXB_4) (6, 7). Therefore, the first step in the LXs synthesis is the production of proinflammatory leukotrienes. LtB_4 is a potent chemotactic agent for neutrophilic leukocytes. It also activates neutrophils in the site of inflammation (8), mobilizes calcium, activates phospholipases, and may induce a bronchoconstriction (9, 10). The production of lipoxins may be initialized also by epithelial cells expressing 15-LOX. The product of this enzyme – 15(S)-hydroperoxyeicosatetraenoic acid (15S-HPETE) – is further taken up by leukocytes where in the presence of 5-LOX, it becomes transformed to LXA_4 (11).

Lipoxins LXA_4 and LXB_4 promote the resolution of inflammation. They inhibit neutrophil chemotaxis (7, 12), eosinophil trafficking and transmigration across postcapillary venules, generation of superoxide anions by neutrophils, and degranulation of azurophilic granules (13), stimulate the clearance

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of apoptotic leukocytes by macrophages, and block natural killer cell cytotoxicity and tumor necrosis factor-alpha release from T cells (13).

A low biosynthetic capacity for lipoxins was found in some chronic inflammatory diseases like severe airway inflammation (13), e.g., chronic asthma (11), cystic fibrosis (12), and chronic liver disease (11).

A study by Vachier et al. measured the concentration of lipoxins in induced sputum of COPD patients (14). In this study, the authors did not find any significant differences in the LXA_4 concentration in COPD patients when compared with control subjects; however, they found the increased levels of lipoxins in patients with mild asthma.

LtB_4 levels both in exhaled breath condensate (EBC) and induced sputum in COPD patients were investigated in several studies. Different authors found the increased levels of LtB_4 in EBC; however, in induced sputum, the data were controversial (10, 15, 16). In the majority of investigations, stable COPD patients were examined. Controversy in results may occur due to activity of the disease in particular study groups. Therefore, we attempted to investigate COPD patients during the exacerbation phase.

To prove our hypothesis of the role of lipoxins in the inflammatory process in COPD, inpatients with COPD exacerbation were recruited for our study, and the concentrations of LtB_4 and LXA_4 were measured in induced sputum and compared with the concentrations of healthy controls. Moreover, the spectrum of inflammatory cells in the sediment of induced sputum was also assessed, and the relationship between this spectrum and the content of proinflammatory and anti-inflammatory eicosanoids was analyzed.

Material and Methods

Subjects. Twenty-four subjects were enrolled into the study. There were 17 inpatients (one woman) with chronic obstructive pulmonary disease (COPD) in the exacerbation stage. Eight received corticoid therapy during hospitalization. COPD patients had a mean number of exacerbations of 1.13±0.09 during the last year. COPD was defined by FEV_1/FVC of <70%, FEV_1 of <80% of predicted, and FEV_1 reversibility <15%. All patients were current or ex-smokers.

Seven healthy control subjects were included into the study. They were ex-smokers or nonsmokers without airway infection within at least last 4 weeks.

Study Design. All subjects had only one visit to our study staff. All subjects were surveyed about their health status: smoking history, corticosteroid use, and duration of exacerbation. Then spirometry with a bronchodilation test was performed. After 15 minutes, postbronchodilation FEV_1 was measured, and an induction of sputum with 4% NaCl solution was initiated.

This case-control study was approved by the Institutional Ethics Committee. Before inclusion in the study, the participants were informed about the study design and possible side effects, and signed written informed consent.

Lung Function. Before the collection of induced sputum, spirometry (spirometer Jaeger MS Pneumo, Germany) with a bronchodilation test was performed for all participants. FEV_1 and FVC were measured according to the European Respiratory Society guidelines (17). Briefly, after the assessment of baseline lung function measurements, all subjects were administered 400 μg of inhaled salbutamol (Ventolin™, GlaxoSmithKline, Riga, Latvia). After 15 minutes, a postbronchodilation spirometry was performed.

Sputum Induction. Sputum induction was performed according to the protocol validated by Pizzichini et al. (18). Shortly, the patients inhaled 4% NaCl from an ultrasonic nebulizer (OMRON NE-U17, OMRON Matsusaka CO, Ltd., Japan). The induction was performed for 3 periods, 5 minutes each. After each step, the examined persons rinsed their mouths and throats with water to minimize contamination with saliva and postnasal drip and then expectorated into a sterile container. The procedure was continued until either a sufficient amount of sputum was obtained (~1 mL) or 3 inhalation periods were over. Before each inhalation period, spirometry was performed. If an FEV_1 fall exceeded 20% of the postbronchodilation value, the procedure was stopped, and a salbutamol inhalation was given to the patient.

Sputum Processing. The sputum samples were processed within 2 or 3 hours after the induction. The volume of induced sputum was measured and mixed with an equal volume of 0.1% dithiothreitol (DL-dithiothreitol, minimum 99% titration, SIGMA-ALDRICH, Inc, St. Louis, USA) that was previously dissolved in Dulbecco's phosphate-buffered saline. The samples then were incubated at 4°C for 15 minutes for complete homogenization. By the end of incubation, the sputum sample was filtered on a 48-μm sterile nylon gauze, and a small amount (20 μg) was used to assess the total cell count and viability, using a standard hemocytometer and the trypan blue dye method. The remaining sample volume was centrifuged at 790*g* for 10 minutes, and the supernatants were collected and stored at –80°C for later analysis. The cell suspension was diluted with phosphate-buffered saline to obtain a concentration of 1×10^6 cells/mL, and the volumes of 100 μL were used for cytospin slides. The dry cytospin slides then were fixed in methanol for 10 minutes, dried,

and stained by the May-Grünwald-Giemsa method (19) for 10 and 15 minutes to estimate the cell count in induced sputum.

Detection of LXA_4 and LtB_4 in Induced Sputum Supernatant. The LXA_4 and LtB_4 concentrations in induced sputum supernatant were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Neogen Corporation, Lexington, USA). The detection limits of the assay for LXA_4 and LtB_4 were 0.02–2 ng/mL and 0.04–4 ng/mL, respectively. The interassay coefficients of variation for LXA_4 and LtB_4 were 17.87% and 15.24%, respectively. The intra-assay coefficients of variation for LXA_4 and LtB_4 were 16.15% and 15.24%, respectively. Each sample was diluted as appropriated and processed in duplicate. The obtained results were extrapolated back to the levels of the original sputum volumes. As the mucous production in COPD patients was more intensive than in healthy persons and the main source of eicosanoids are leukocytes, their content in sputum was also expressed per one leukocyte (Ne+Mo+Eo+Ly)

Statistical Analysis. Data processing was done using the software Statistica 7.0 (StatSoft, Inc., USA). Data were expressed as mean with 95% confidence interval. The LXA_4 and LtB_4 content in supernatant was extrapolated to the whole sputum. If the data did not conform to a normal distribution, their compliance to a logarithmical normal distribution was assayed. If the data conformed to the latter distribution, the logarithmic data transformation was performed: $y'=\log_{10}y$ or $y'=\log_{10}(y+1)$ (the value of variable was between 0–1) (20). If the data were with a lesser degree of asymmetry, the square root transformation was used: $y'=\sqrt{y}$ or $y'=\sqrt{y}+0.5$ (value of variable was between 0–1) (20). The data of relative cell counts were processed with an arc-sine transformation: $y'=\arcsin\sqrt{(y/100)}$ (20). Statistical analysis was carried out by the analysis of variance (one-factor ANOVA). If the data were previously transformed, an opposite transformation of obtained average data and mean upper and lower limits of 95% confidence interval then were made, i.e., $y=10^{y'}$ or $y=10^{y'}-1$, $y=y'^2$ or $y=y'^2-0.5$, and $y=10(\sin y')^2$ (20).

A *P* value of <0.05 was considered as statistically significant.

Results

Table gives the characteristics of COPD patients and healthy controls. The patients with COPD were significantly older than the control patients ($P=0.046$). COPD patients had significantly lower FEV_1 and FEV_1/FVC values compared with the control group ($P<0.0001$). Patients with COPD had a greater pack-year smoking history than control persons ($P=0.0001$).

Table. Characteristics of the Study Population

Characteristic	COPD Patients	Healthy Controls	*P*
	n=17	n=7	
Gender, n Men/women	16/1	4/3	NS
Age, years	61.18±2.32	52.71±2.61	0.046
Smoking history, pack-years	34.56±4.48 (n=16)	2.43±1.23 (n=3)	0.0001
Ex-smokers	3 (n=16)	3 (n=3)	
FEV_1, % predicted	47.77±5.39	102.60±3.90	<0.0001
Postbronchodilation FEV_1, %	51.38±5.82	106.1±4.16	<0.0001
Reversibility, %	3.01±0.83	3.57±1.53	NS
Ratio FEV_1/FVC, %	53.88±2.31	83.00±1.72	<0.0001

Vales are mean ± 95% confidence interval.
NS, not significant.

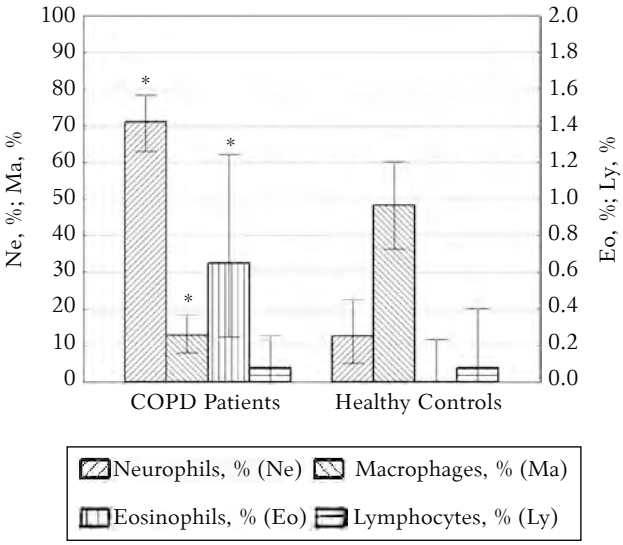

Fig. 1. The relative counts of neutrophils, macrophages, eosinophils, and lymphocytes in induced sputum of COPD patients (n=17) and healthy controls (n=7)

Bars represent 95% confidence interval of mean value.
*$P<0.05$.

In COPD patients, the relative neutrophil and eosinophil counts were significantly higher than in healthy controls. The relative macrophage count was lower in the sputum of COPD patients compared with healthy controls (Fig. 1).

The LXA_4 concentration in induced sputum as well as the LXA_4 content per one leukocyte were significantly lower in COPD patients compared with healthy controls ($P=0.0094$ and $P=0.0004$, respectively) (Figs. 2A and 2B). The LtB_4 concentration in induced sputum did not significantly differ between both the examined groups (Fig. 2C). However, if the LtB_4 content was expressed per one leukocyte, the difference became significant, showing a reduced value in COPD patients ($P=0.0367$) (Fig. 2D).

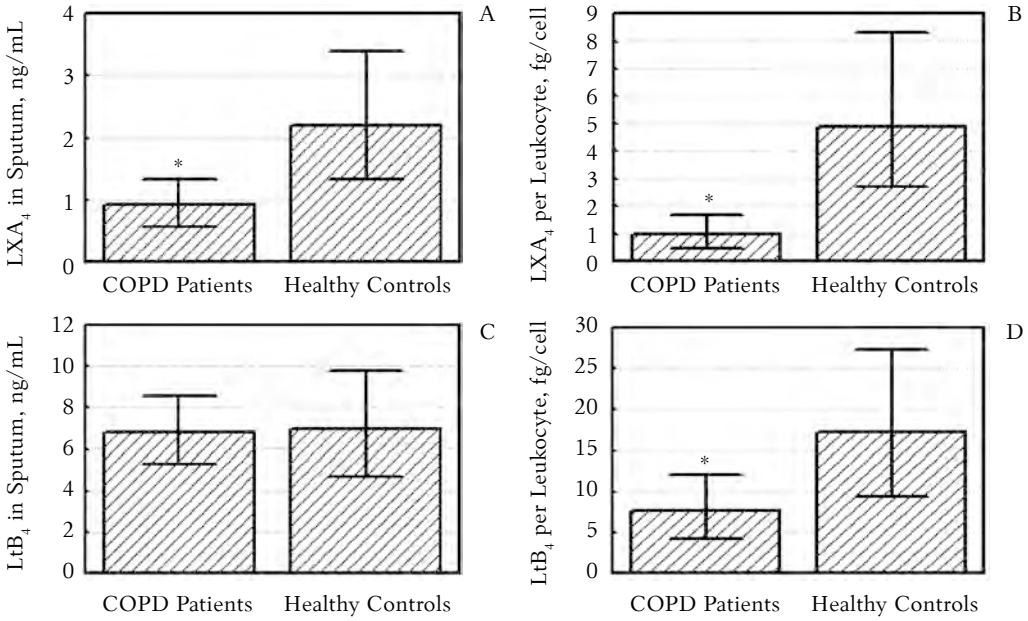

Fig. 2. LXA_4 and LtB_4 concentrations and their concentrations per one leukocyte in induced sputum of COPD patients and healthy controls

A, LXA_4 concentration; B, LXA_4 concentration per one leukocyte; C, LtB_4 concentration; and D, LtB_4 concentration per one leukocyte.
Bars represent 95% confidence interval of mean value. *P<0.05.

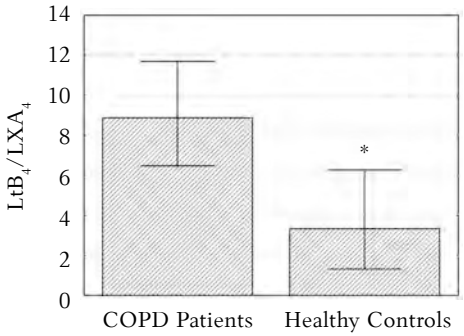

Fig. 3. The ratio of leukotriene B_4/lipoxin A_4 (LtB_4/LXA_4) in induced sputum of COPD patients (n=17) and healthy controls (n=7)

Bars represent 95% confidence interval of mean value. *P<0.05.

The LtB_4/LXA_4 ratio was almost 3 times higher in the COPD group compared with the control group (P=0.0071) (Fig. 3).

To evaluate the effect of eicosanoids on a leukocyte spectrum in sputum, the control group was merged with the COPD group, and correlation between particular leukocyte count and eicosanoid concentration in induced sputum was assessed. A significant negative correlation between the LXA_4 concentration and the relative sputum neutrophil count (r^2=0.232, P=0.0017) and between the LtB_4/LXA_4 ratio in sputum and the relative macrophage count (r^2=0.174, P=0.0428) was documented. No significant correlation was found between the relative neutrophil count and LtB_4 concentration or between the relative neutrophil count and the LtB_4/LXA_4 ratio. No correlation was also found between the LtB_4/LXA_4 ratio and the relative eosinophil count in sputum.

To evaluate the possible effect of smoking on our results, COPD patients were divided in smokers and nonsmokers, and the eicosanoid content was compared. No significant difference was found between the groups. Similarly, the impact of corticosteroid treatment on our results was examined by dividing COPD patients into steroid users and steroid-naïve patients. No significant differences in the eicosanoid content in sputum were found.

Discussion

The obtained results have shown that the induced sputum of patients with exacerbated COPD had significantly lower levels of LXA_4 compared with healthy subjects. This difference was even more marked if the values were expressed as the LXA_4 content per one sputum leukocyte. Unexpectedly, no elevated LtB_4 concentration in the sputum of COPD patients was found. Even more, if the amount of LtB_4 was expressed per one leukocyte, the COPD group had a significantly lower LtB_4 value than the control group. However, despite low LtB_4 levels, the LtB_4/LXA_4 ratio was significantly higher in the COPD group than the control group indicating a proinflammatory pattern of eicosanoid release.

Corhay et al. also reported no difference in the LtB_4 levels between COPD patients and control subjects (15). This was explained by possible contamination of the sputum specimens with saliva containing the high amounts of LtB_4 in healthy people. In our study, salivary contamination was prevented by mouth rinsing before sputum expectoration, and the level of salivary contamination was judged by assessing squamous epithelial cell count in the specimen. Therefore, this explanation was not considered as valid in our case.

A study by Bhavsar et al. (21) examined macrophages obtained from asthmatic patients and found a decreased basal production of both LtB_4 and LXA_4 in patients with a severe disease as compared with patients with a mild disease and healthy controls.

To understand the reason for such diverse results reported by different authors, it is important to take into account the kinetics of an inflammatory process. In a murine model of zymosan-induced peritonitis, the LtB_4 level in peritoneal lavage fluid peaked within the first 4 hours after zymosan injection and then gradually dropped to the basic level within 24 hours. This was followed by neutrophil immigration into the peritoneal cavity that reached the peak after 24 hours and lasted up to 72 hours. The LXA_4 level also peaked after 4 hours, then decreased to one-third of the initial level, and remained at this level for more than 72 hours. It paralleled with the accumulation of mononuclear cells (22). In an experimental mouce model of lung inflammation induced by an intratracheal administration of endotoxin, the neutrophil count in alveolar lavage fluid peaked after 4 days (23).

To our knowledge, the only data on inflammation kinetics in human lungs can be obtained from the study by Crooks et al. (24). The authors analyzed the sputum samples for LtB_4 levels and myeloperoxidase activity in COPD patients each day during the exacerbation. After the beginning of antibiotic treatment, the LtB_4 level decreased progressively, reaching the stable level on the fifth day of treatment. The myeloperoxidase level, reflecting the gross activity of neutrophils, dropped 5-fold on the fifth day of treatment and reached the stable level after 2 weeks.

In our study, induced sputum could not be examined during the first days after patient's admission to hospital due to severity of their condition. On the average, sputum was induced on the seventh day of exacerbation. At this time, according to the observations of Crooks et al. (24), the LtB_4 levels decreased approximately 10-fold compared with the first day reaching lower levels than observed in the same patients during the stable stage. Evidently, low levels of LtB_4 observed in our study reflected this postexpression period. Despite low LtB_4 level, the LtB_4/LXA_4 ratio was greater than in control subjects. It indicated conditions promoting the neutrophil persistence in an inflammation area. The LXA_4 levels at measured period were significantly lower than those in healthy individuals. Based on experimental animal data, at this time we could expect the elevated levels of lipoxins in induced sputum.

To demonstrate the functional relationship between lipoxins and leukocyte spectrum, the correlation between these indices in the general group that consisted of healthy individuals without inflammation in airways and persons with inflammation of different severity was examined. A significant inverse correlation between the LXA_4 concentration and the relative neutrophil count in induced sputum clearly demonstrated a suppressive role of lipoxins in the inflammatory process of airways. A positive correlation between the LXA_4 concentration and the monocyte count supported the role of lipoxins in nonphlogistic macrophage recruitment in a repair process (25). No correlation between the proinflammatory LtB_4 level and the neutrophil found in our general group could be explained by sputum examination in the late stage of exacerbation when leukotriene levels were decreased.

A drop in LtB_4 levels during the course of inflammation is explained by its fast inactivation. LtB_4 is rapidly metabolized in vivo by several pathways. One of the major routes is ω-oxidation. Neutrophils and other cells express cytochome P-450 that carries out ω-oxidation of LtB_4 to form 20-hydroxy-LtB_4 and further 20-carboxy-LtB_4 (26, 27). An alternative conversion pathway of LtB_4 is initiated by a reduction of LtB_4 followed by ω-hydroxylation (28). In induced sputum of COPD patients, inflammatory cells, including neutrophils, are in a much greater amount than in induced sputum of healthy persons, and therefore, the modification of LtB_4 by neutrophils could occur more rapidly. This could explain lower LtB_4 levels in COPD patients compared with healthy controls.

In general, the sputum concentrations of LXA_4 and LtB_4 in our study were higher than reported by other investigators. It could be explained by the different methods of sputum processing. In our study, dithiothreitol (DTT) was added to sputum at the very beginning of the sputum processing, and the LtB_4 content was measured in the presence of DTT. As DTT cleaves disulphide bonds, it could influence the activity of enzymes involved in the metabolism of arachidonic acid (29). However, higher concentrations of eicosanoids observed in our study could not influence the differences observed between COPD patients and healthy persons.

In our study, 8 COPD patients received corticosteroid treatment. It is known that corticosteroid use

can influence eicosanoid release from inflammatory cells. Bhavsar et al. (21) have demonstrated that alveolar macrophages obtained from BAL fluid of healthy persons and patients with asthma decrease lipopolysaccharide-induced LtB_4 and LXA_4 release in the presence of dexamethasone. However, this suppressor effect was strongly dampened in patients with severe asthma (21). No significant difference in the LtB_4 and LXA_4 levels between our steroid users or steroid-naïve patients was found.

Steroid insensitivity of COPD patients is a widely described phenomenon (30). It is caused by histonedeacetylase (HDAC2) dysfunction in COPD patients (31). HDAC2 is an intranuclear enzyme that suppresses inflammatory gene expression. One of the causes leading HDAC2 dysfunction and corticosteroid insensitivity is S-nitrosylation of HDAC2. Interestingly, S-nitrosylation of HDAC2 in alveolar macrophages occurs from exposure to cigarette smoke that is a primary cause of COPD. Denitrosylation of HDAC2 by targeting Nrf2 restores glucocorticosteroid sensitivity in macrophages from COPD patients (32). All our COPD patients, except 2 ex-smokers, were current smokers.

One of the potential limitations of our study could be the presence of 2 ex-smokers in the COPD group. However, they were not excluded from the study group because there is strong evidence that smoking cessation does not stop a chronic inflammatory process in the lungs of COPD patients (33, 34).

Recently, the prospective study involving ex-smokers has demonstrated that 4 years after smoking cessation, induced sputum still had the increased levels of mediators of inflammation (myeloperoxidase, LtB_4, IL-8, monocyte chemoattractant protein-1, matrix metalloprotease-9), which was associated with a significant progression of COPD on a chest CT scan (35).

An increasing body of scientific data in recent years has led to new understanding of an inflammation process. Instead of a previous concept of inflammation as a passive process that terminates when the clearance of an inflamed area from pathogens or damaged cells is completed, a new concept of active promotion of resolution and termination of inflammation is defined (36). Signals that promote the resolution induce nonphlogistic activity of macrophages including the phagocytosis of apoptotic neutrophils, reduce vascular permeability, and return parenchymal cells to a noninflammatory state. Lipoxins are among these resolution-promoting substances, and this study shows that the persistence of inflammation present in COPD is explained at least by the insufficiency of this signaling system. Stable analogues of lipoxins that could serve as resolution-promoting drugs are under investigation (37, 38). COPD could become one of chronic inflammatory diseases that will be treated with such kind of drugs.

Conclusions

The obtained data show the suppressed production of LXA_4 and the elevated LtB_4/LXA_4 ratio in induced sputum of patients with chronic obstructive pulmonary disease during the late phase of exacerbation. This pattern of inflammatory response is different from that observed in experimental animal models and demonstrates a proinflammatory imbalance in chronic obstructive pulmonary disease. The correction of a balance between proinflammatory and anti-inflammatory eicosanoids by the administration of stable analogues of lipoxins could improve the treatment of chronic obstructive pulmonary disease in the future.

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Statement of Conflict of Interest

The authors state no conflict of interest.

References

1. Stockley RA, Mannino D, Barnes PJ. Burden and pathogenesis of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2009;6:524-6.
2. Garcia-Aymerich J, Agusti A, Barbera JA, Belda J, Farrero E, Ferrer A, et al. Phenotypic heterogeneity of chronic obstructive pulmonary disease. Arch Bronconeumol 2009; 45(3): 133-42.
3. Singh S. Chronic obstructive pulmonary disease. Curr Anaest Crit Care 2003;14:74-80.
4. Sutherland ER, Martin RJ. Airway inflammation in chronic obstructive pulmonary disease: comparisons with asthma. J Allergy Clin Immunol 2003;112:819-27.
5. Simoni-Wastila L, Blanshette CM, Qian J, Yang HK, Zhao L, Zuckerman IH, et al. Burden of chronic obstructive pulmonary disease in medicare beneficiaries residing in long-term care facilities. Am J Geriatr Pharmacother 2009;7(5): 262-70.
6. Chiang N, Arita M, Serhan CN. Anti-inflammatory circuitry: lipoxin, aspirin-triggered lipoxins and their receptor ALX. Prostaglandins Leukot Essent Fatty Acids 2005;73: 163-77.
7. Ryan A, Godson C. Lipoxins: regulators of resolution. Curr Opin in Pharmacol 2010;10:166-72.
8. Widegren H, Andersson M, Borgeat P, Flamand L, Johnston S, Greiff L. LtB4 increases nasal neutrophil activity and conditions neutrophils to exert antiviral effects. Respir Med 2011;105(7):997-1006.
9. Peters-Golden M, Canetti C, Mancuso P, Coffey MJ. Leukotrienes: underappreciated mediators of innate immune responses. J Immunol 2005;174:589-94.
10. Kostikas K, Gaga M, Papatheodorou G, Karamanis T, Orphanidou D, Loukides S. Leukotiene B4 in exhaled breath condensate and sputum supernatant in patients with COPD and asthma. Chest 2005;127:1553-9.

11. McMahon B, Godson C. Lipoxins: endogenous regulators of inflammation. Am J Physiol Renal Physiol 2004;286: F189-201.
12. Karp CL, Flick LM, Yang R, Uddin J, Petasis NA. Cystic fibrosis and lipoxins. Prostaglandins Leukot Essent Fatty Acids 2005;73:263-70.
13. Haworth O, Levy BD. Endogenous lipid mediators in the resolution of airway inflammation. Eur Respir J 2007;30: 980-92.
14. Vachier I, Bonnans C, Chavis C, Farce M, Godard P, Bousquet J, et al. Severe asthma is associated with a loss of LX4, an endogenous anti-inflammatory compound. J Allergy Clin Immunol 2005;115:55-60.
15. Corhay JL, Henket M, Nguyen D, Duysinx B, Sele J, Louis R. Leukotriene B4 contributes to exhaled breath condensate and sputum neutrophil chemotaxis in COPD. Chest 2009; 136:1047-54.
16. Ko FWS, Leung TF, Wong GWK, Ngai J, To KW, Ng S, et al. Measurement of tumour necrosis factor-alpha, leukotriene B4, and interleukin 8 in exhaled breath condensate in patients with acute exacerbations of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2009; 4:79-86.
17. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26:319-38.
18. Pizzichini E, Pizzichiani MMM, Leigh R, Djukanovic R, Sterk PJ. Safety of sputum induction. Eur Respir J Suppl 2002;37:9s-18s.
19. Amer M, Elnasser TA, El Hagger S, Mostafa T, Abdel-Malak G, Zohdy W. May-Grunwald-Giemse stain for detection of spermatogenic cells in the ejaculate: a simple predictive parameter for successful testicular sperm retrieval. Hum Reprod 2001;16(7):1427-32.
20. Zar JH. Biostatistical analysis. In: Snavely SL, editor. 4th ed. New Jersey: Prentice-Hall, Inc. Simon & Schuster/AViacom Company; 1999. p. 275, 278, 353.
21. Bhavsar PK, Levy BD, Hew MJ, Pfeffer MA, Kazani S, Israel E, et al. Corticosteroid suppression of lipoxin A_4 and leukotriene B4 from alveolar macrophages in severe asthma. Respir Res 2010;11:71-9.
22. Chiang N, Schwab JM, Fredman G, Kasuga K, Gelman S, Serhan CN. Anesthetics impact the resolution of inflammation. PLoS One 2008;3(4):e1879.
23. D'Alessio FR, Tsushima K, Aggarwal NR, West EE, Willett MH, Britos MF, et al. CD4+CD25+Foxp3+Tregs resolve experimental lung injury in mice and are present in humans with acute lung injury. J Clin Invest 2009;119(10):2898 913.
24. Crooks SW, Bayley DL, Hill SL, Stockley RA. Bronchial inflammation in acute bacterial exacerbation of chronic bronchitis: the role of leukotriene B4. Eur Respir J 2000;15:274-80.
25. Serhan CN, Brain SD, Buckley CD, Gilroy DW, Haslett C, O'Neill LAJ, et al. Resolution of inflammation: state of the art, definitions and terms. FASEB J 2007;21(2):325-32.
26. Pettipher ER, Salter ED, Breslow R, Raycroft L, Showell HJ. Specific inhibition of leukotriene B4 (LtB4)-induced neutrophil emigration by 20-hydroxy LtB4: implications for the regulation of inflammatory responses. Br J Pharmacol 1993;110(1):423-7.
27. Hankin JA, Murphy RC. The metabolism of leukotriene B4 in Lewis lung carcinoma porcine kidney cells. Am J Respir Crit Care Med 2000;161:S81-7.
28. Brom J, Schönfeld W, König W. Metabolism of leukotriene B_4 by activated human polymorphonuclear granulocytes. Immunology 1988;64(3):509-18.
29. Kelly MM, Wightman PD, Berlyne GS, Leigh R, Morris MM, Hargreave FE. Repeability of leukotriene measurements in induced sputum. Eur Respir J 2000;16:220S.
30. Barnes PJ. Role of HDAC2 in the pathophysiology of COPD. Annu Rev Physiol 2009;71:451-64.
31. Isajevs S, Taivans I, Kopeika U, Strazda G. Inflammatory cells and HDAC2 expression in peripheral airways of COPD patients. Mod Pathol 2006;19(Suppl 3):167-8.
32. Malhotra D, Thimmulappa RK, Mercado N, Ito K, Kombairaju P, Kumar S, et al. Denitrosylation of HDAC2 by targeting Nrf2 restores glucocorticosteroid sensitivity in macrophages from COPD patients. J Clin Invest 2011;121(11):4289-302.
33. Morales SA, Dreyse DJ, Díaz PO, Saldías PF, Carrasco M, Lisboa BC. Systemic inflammation among stable ex smokers with chronic obstructive pulmonary disease. Rev Med Chil 2010;138(8):957-64.
34. Papaioannou AI, Loukides S, Minas M, Kontogianni K, Bakakos P, Gourgoulianis KI, et al. Exhaled breath condensate pH as a biomarker of COPD severity in ex-smokers. Respir Res 2011;12:67-73.
35. Miller M, Cho JY, Pham A, Friedman PJ, Ramsdell J, Broide DH. Persistent airway inflammation and emphysema progression on CT scan in ex-smokers observed for 4 years. Chest 2011;139(6):1380-7.
36. Shields AM, Panayi GS, Corrigall VM. Resolution-associated molecular patterns (RAMP): RAMParts defending immunological homeostasis? Clin Exp Immunol 2011;165(3): 292-300.
37. Levy BD, Lukacs NW, Berlin AA, Schmidt B, Guilford WJ, Serhan CN, et al. Lipoxin A4 stable analogs reduce allergic airway responses via mechanisms distinct from CysLT1 receptor antagonism. FASEB J 2007;21:3877-84.
38. Petasis NA, Keledjian R, Sun YP, Nagulapalli KC, Tjonahen E, Yang R, et al. Design and synthesis of benzo-lipoxin A4 analogs with enhanced stability and potent anti-inflammatory properties. Bioorg Med Chem Lett 2008;18(4):1382-7.

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