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Measurement of bronchoconstrictive eicosanoids in chronic obstructive pulmonary disease

Pomiar bronchospastycznych eikozanoidów w przewlekłej obturacyjnej chorobie płuc

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Abstract

Introduction: The aim of the study was to evaluate the concentration of 9a11b prostaglandin F2, a stable metabolite of prostaglandin D2 (PGD2) and leukotriene E4 (LTE4), in patients with stable and exacerbated chronic obstructive pulmonary disease (COPD).

Material and methods: The study included 29 COPD patients aged 73 ± 8.34 years, with mean FEV1 = 48.64 ± 15.75% of predicted normal value, and 29 healthy controls aged 57.48 ± 10.86 years, with mean FEV1 = 97.17 ± 13.81% of predicted normal value. Urine and blood samples were taken from COPD patients during exacerbation and in the stable phase of the disease; LTE4 was measured in urine using commercial enzyme immunoassay (EIA), and 9a11b prostaglandin F2 (9a11bPGF2), a stable metabolite of PGD2, was measured in blood and urine using GC/MS.

Results: Urine concentrations of LTE4 in urine (677.15 vs. 436.4 pg/mg of creatinine; $p = 0.035$) and serum levels of 9a11bPGF2 (5.35 vs. 3.07 pg/ml; $p = 0.007$) were significantly higher in patients with exacerbated COPD than in the control group. There was no difference in LTE4 levels in urine and 9a11bPGF2 in serum between exacerbated and stable COPD. The urinary 9a11bPGF2 concentration did not differ between the studied groups. We found a positive correlation between smoking history and urine LTE4 level ($r = 0.395$; $p = 0.002$) as well as blood 9a11bPGF2 concentration ($r = 0.603$; $p = 0.001$) in COPD patients.

Conclusions: Urine levels of 9a11bPGF2 and LTE4 did not differ between the stable COPD group and the control group. There were no differences between urine LTE4 levels and blood and urine 9a11bPGF2 levels between exacerbated and stable COPD. Finally, LTE4 concentration in urine and 9a11bPGF2 in blood were significantly higher in exacerbated COPD patients than in the control group.

Key words: COPD, LTE4, PGD2, bronchoconstrictive eicosanoids

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Introduction

Chronic obstructive pulmonary disease (COPD) and bronchial asthma have a common denominator of chronic inflammatory reaction in the bronchial tree and lung parenchyma, resulting in decreased bronchial air flow, which in COPD may be partially irreversible [1–3].

Differences in cellular composition, underlying mechanisms, distribution of lesions, and involved mediators in the course of remodelling of the respiratory tract in respective diseases are well known. These particularities are most pronounced, for example, in non-smoking patients with moderate asthma as compared to smoking COPD patients with moderately advanced disease. The more advanced both diseases become, the less sharp is the difference between them; therefore, separation between these entities may seem only arbitrary.

Severe asthma with low reversibility of bronchoconstriction after bronchodilator administration and resistance to systemic glucocorticoids is marked by increased numbers of neutrophil granulocytes in bronchial mucosa samples and in bronchioloalveolar lavage (BAL), with increased proportion of CD8(+) lymphocytes observed in autopsy samples [4–7]. In some COPD patients, histological and cytological findings demonstrate eosinophilia in sputum, BAL, and bronchial mucosa samples. These inflammatory cells are characteristic for asthma, and their numbers increase in COPD exacerbations, which coincides with increasing levels of eosinophil cationic protein (ECP) and increased expression of chemotactic factors [8–10]. Bronchial mucosa samples from COPD patients also show mast cell infiltration, usually perceived as hallmarks of allergic diseases. Mast cell infiltration decreases under corticosteroid therapy, which coincides with regression of symptoms [9]. The role of mast cells in the development of bronchial obturation is related to their enzymatic constitution, as proteases (tryptase, chymase, elastase) synthesized by mast cell damage to bronchial wall components, inducing hyperreactivity and increasing mucus secretion. On the other hand, mast cells can synthesize and secrete the most potent bronchoconstrictors, including cysteinyl leukotrienes and prostaglandin D2 [12–15]. Therefore, the impact of eosinophils and mast cells, as well as their mediators, on the development of airway obturation in COPD patients can be of great interest.

The aim of the study was to evaluate concentrations of leukotriene E4 (LTE4) and 9a11bPGF2, a stable metabolite of prostaglandin D2, in patients with COPD during stable disease and exacerbations.

Material and methods

The study group included 29 patients with COPD (8 women, 21 men), aged 54–86 (mean age 73 ± 8.34) years. Mean value of forced expiratory volume in one second (FEV1) was 1.22 ± 0.45 L in stable disease (48.64 ± 15.75% of predicted normal value). In phases of disease exacerbation, FEV1 values were significantly lower compared to readings from stable disease in the same patients ($p = 0.003$). Eleven patients (37.93%) had moderate disease, 15 patients had severe disease (51.72%), and 3 persons suffered from a very severe form of COPD (10.34%).

The control group included 29 persons (15 women, 14 men), aged 56–80 (mean age 57.48 ± 10.86) years. There were both non-smokers and smoking persons in the control group, but none of them had symptoms of COPD, asthma, or allergy. All patients had normal serum IgE levels and numbers of blood eosinophils. Mean FEV1 value was 2.71 ± 0.61 l (97.17 ± 13.81% of predicted normal value). Exclusion criteria were signs of systemic mastocytosis, unstable angina pectoris, or heart infarction as these entities are marked by strong mast cell activation.

Patients with known COPD were qualified for treatment within the first three days of exacerbation, which was defined as suddenly increased respiratory symptoms (dyspnoea, cough, increased sputum production) as compared to each patient's stable state, with necessity of treatment modification in an outpatient clinic or in hospital conditions. Diagnosis and classification of COPD exacerbation was based on the criteria described by Anthonisen et al. [16]. Three categories of exacerbation were assigned, depending on the number and type of observed symptoms: mild, moderate, and severe. On inclusion, spirometry was performed and FEV1 was used as a denominator of exacerbation severity. Furthermore, blood and urine samples were collected for evaluation of serum and urine 9a11bPGF2 levels by gas chromatography/mass spectrometry (GC/MS; Hewlett Packard, Paolo Alto, USA); urine LTE4 levels by enzyme-linked immunosorbent assay (ELISA; Cayman Chemicals, AnnArbor, USA); absolute blood eosinophil count by microscopy using a Burker chamber; and total serum IgE content by nephelometry (Dade Behring, Newark, USA). Samples were collected before onset of therapy for COPD exacerbation. Before exacerbation, COPD patients were treated with long acting beta2-adrenoreceptor blockers; 48.28% of patients received inhaled steroids, and 41.38% of persons had systemic steroids with a

mean dose of 5.55 mg calculated for methylprednisolone dose.

When a stable state was reached, approximately 2 months after exacerbation, spirometry was performed again for evaluation of disease severity, and biochemical tests were again performed. All patients with stable disease were treated with long-acting beta2-adrenoreceptor blockers; 65.52% of patients received inhaled steroids, and 27.59% of persons took oral steroids, with a mean dose of 4.7 mg calculated for methylprednisolone dose (table 1).

In the control group, spirometry was performed once, followed by urine and serum marker measurement as described above.

Results

Analysis of urine samples from COPD patients showed significantly higher levels of LTE4 in exacerbation phase as compared to healthy controls (677.15 vs. 436.3 pg/mg creatinine; $p = 0.035$).

In stable COPD phase, urine LTE4 levels were insignificantly lower as compared to exacerbation readings, but these were at the same time higher than in healthy subjects (table 2).

Serum levels of 9a11bPGF2 in patients with exacerbated COPD were significantly higher than in the control group (5.35 vs. 3.07 pg/ml; $p = 0.007$).

The level of the analysed PGD2 metabolite was higher under COPD exacerbation than in stable disease. Differences between 9a11bPGF2 readings in these two disease stages were almost significant (5.35 vs. 3.81 pg/ml; $p = 0.068$). Mean serum concentration of 9a11bPGF2 in COPD stable patients and in healthy controls did not differ significantly (3.81 vs. 3.07 pg/ml; $p > 0.05$) (table 3).

There were no significant differences between mean urine PGD2 metabolite levels in exacerbated and stable COPD (0.46 vs. 0.44 ng/mg creatinine; $p > 0.05$). Mean urine levels of 9a11bPGF2 in COPD patients, both with exacerbated and stable disease, were not significantly different from values found in healthy subjects (0.46 vs. 0.57 ng/mg creatinine; $p > 0.05$ and 0.44 vs. 0.57 ng/mg creatinine; $p > 0.05$) (table 4).

Correlation was found between smoking habit (measured in pack-years) and urine LTE4 levels, both in stable COPD ($r = 0.574$; $p = 0.002$) and in exacerbated disease ($r = 0.395$; $p = 0.041$), as well as between smoking habit and serum 9a11bPGF2 level in exacerbated COPD ($r = 0.603$; $p = 0.001$).

Discussion

Current research on the mechanisms underlying allergic diseases, including bronchial asthma, focuses on mast cells and mediators secreted by them, including prostaglandin D2 and cysteinyl leukotrienes. The role of these cells is also investigated in COPD. Histopathological pictures of bronchial mucosa samples from patients with chronic bronchitis reveal an increased population of mast cells within the surface epithelium and in submucosal glands [17]. Lung tissue from tobacco

Table 1. Characteristics of the study group and control group

	Study group	Control group	
Number of patients	n	29	29
Age (years)	n ± SD	71.38 ± 8.34	57.48 ± 10.86
Women	n (%)	8 (27.59)	15 (51.72)
FEV1 (L)	n ± SD	1.22 ± 0.45	2.71± 0.61
FEV1 (%)	n ± SD	48.64 ± 15.75	97.17 ± 13.81
FVC (L)	n ± SD	2.27 ± 0.71	3.47 ± 0.73
FVC (%)	n ± SD	70.74 ± 15.72	102.3 ± 15.10
Smokers	n (%)	29 (100)	22 (75.86)
Current smokers	n (%)	20 (68.97)	9 (31.03)
Ex-smokers	n (%)	9 (31.03)	13 (44.83)
IgE [IU/ml]	n ± SD	66.32 ± 70.03	47.92 ± 32.49
Eosinophils [n/il]	n ± SD	232 ± 169	188.45 ± 106.09
Systemic steroid therapy (GCS)	n (%)	12 (41.38)/8 (27.59)	0 (0)
Inhaled steroids	n (%)	14 (48.28)/19 (65.52)	0 (0)

FEV1 = forced expiratory volume in one second; GCS = glucocorticoids; IgE = immunoglobulin E; SD = standard deviation

Table 2. Leukotriene E4 (LTE4) concentrations in urine during exacerbation of chronic obstructive pulmonary disease (COPD) in stable COPD and in healthy controls

LTE4 [pg/mg creatinine]	COPD exacerbation n = 27	Stable COPD n = 29	Control group n = 29	Exacerbated vs. stable	p Exacerbated vs. stable	Stable vs. control group
Mean ± SD	677.15 ± 543	613.31 ± 719	436.3 ± 243	0.62	0.035	0.70
Median	559	373	400			
Min & max	(103–2,646)	(56–3,802)	(93–1117)			

SD = standard deviation

Table 3. Serum 9a11bPGF2 concentration during exacerbation of chronic obstructive pulmonary disease (COPD), in stable COPD and in healthy controls

9a11bPGF2 [pg/ml]	COPD exacerbation n = 27	Stable COPD n = 29	Control group n = 29	Exacerbated vs. stable	p Exacerbated vs. stable	Stable vs. control group
Mean ± SD	5.35 ± 5.35	3.81 ± 1.99	3.07 ± 1.05	0.068	0.007	0.225
Median	3.6	3.1	2.9			
Min & max	(1.2–30.2)	(1.0–9.5)	(1.2–5,6)			

SD = standard deviation

smokers in resection specimens due to cancer shows the presence of mast cells in the epithelium of small bronchi [18]. The role of mast cells in COPD pathogenesis may also be implied when observing effects of inhaled steroids on the frequency of disease exacerbations. Giżycki et al. treated their patient for 3 months with 1000 ìg fluticasone propionate, and afterwards observed significantly decreased numbers of mucosal mast cells in COPD patients, with clinical findings of reduced cough intensity, decreased amount of produced sputum, and fewer exacerbation episodes [11].

Prostaglandin D2 is currently viewed as the most sensitive and specific marker of inflammatory reaction with a mastocytic component. Serum and urine levels of 9a11bPGF2, a stable metabolite of PGD2, have been measured in patients under COPD exacerbation and stable disease. Significantly higher concentrations of 9a11bPGF2 were found in serum under exacerbation as compared to those found in the control group. The differences between 9a11bPGF2 levels in exacerbated and stable COPD were, however, insignificant (p = 0.068), which may be explained by the low number of patients. Serum levels of PGD2 metabolite in control subjects and stable COPD were not different, and neither were urine levels of the same substance.

Few reports concerning PGD2 or its metabolites have been published until now; therefore, there is only sparse data to compare with. Montuschi et al. found similar levels of PGD2 metabolite, metoxyPGD2, in exhaled air in COPD patients and healthy controls [19]. Of note, prostaglandin levels could be measured in only half of the studied persons.

In the presented study, significantly higher LTE4 levels in urine were observed in exacerbated COPD patients as compared to healthy persons, but no such differences could be noted between COPD in stable and exacerbated phase. These findings are in accordance with the results published by Micheletto et al., who compared urine LTE4 concentrations in stable COPD and exacerbated disease, in healthy controls and in patients with mild atopic asthma [20]. Leukotriene synthesis in COPD exacerbation and stable phase was assessed in two groups of patients independently, which differentiates the cited report from our study. The authors observed significantly higher levels of urine LTE4 in exacerbated COPD and mild asthma when compared to the control group. There were no major differences in LTE4 levels between healthy subjects and patients with stable COPD nor between COPD patients with stable or exacerbated disease. Of note, mean LTE4 levels during COPD exacerbations were similar to the levels observed in patients with mild asthma and history of atopic disease.

A lack of significant differences in LTE4 synthesis between COPD patients and healthy controls was also described by Mierzejewska et al., who

Table 4. Urine 9a11bPGF2 concentration during exacerbation of chronic obstructive pulmonary disease (COPD), in stable COPD and in healthy controls

9a11bPGF2 [pg/ml]	COPD exacerbation n = 27	Stable COPD n = 29	Control group n = 29	Exacerbated vs. stable	p Exacerbated vs. stable	Stable vs. control group
Mean ± SD	0.49 ± 0.27	0.50 ± 0.35	0.62 ± 0.37	0.844	0.181	0.113
Median	0.46	0.44	0.57			
Min & max	(0.1–1.12)	(0.13–1.59)	(0.07–1.56)			

SD = standard deviationa

analysed both urine samples and exhaled air [21]. Shindo et al. found, however, significantly higher serum LTE4 levels under COPD exacerbation when compared to stable disease [22]. These findings differ from the conclusions of the presented study but can possibly be explained by different measurement techniques and examination of serum, and not urine samples. In the cited report, LTE4 levels in stable disease were similar to those found in healthy patients.

When coparing the presented results with those from another study from the same centre, it should be noted that urine LTE4 levels were similar in our group and in patients with stable allergic asthma, investigated by Bochenek et al., who analysed urine samples in stable disease and after allergen provocation [23]. Baseline urine LTE4 levels in asthmatic patients were similar to the ones we found in patients with exacerbated COPD. However, these levels increased by almost 2.5 times after allergen provocation (which is a supposed counterpart of disease exacerbation), reaching levels not noted in COPD.

In the presented study, a correlation was found between the number of smoked cigarettes (expressed in pack-years) and urine LTE4 levels, both in stable and exacerbated COPD. This finding indirectly supports data from literature concerning cell composition in the bronchial tree in smokers. Amin et al. observed significantly higher numbers of mast cells and eosinophils in mucosal samples from asymptomatic smokers when compared to non-smoking persons. Both of these cell types play a major role in synthesis of cysteinyl leukotrienes [24]. Moreover, increased expression of tenascin and laminin as well as discontinuity of respiratory epithelium could be found microscopically. Furthermore, lung parenchyma in specimens resected from smokers diagnosed with cancer showed the presence of mast cells in the epithelium of small bronchi, which suggests a relationship between mast cell infiltration and smoking, and thus also in COPD development [25].

Increased LTE4 synthesis in COPD exacerbation seems to correlate with increased inflammation, although not equally marked in asthma exacerbation. Escalating intensity of inflammatory reaction results in increased local influx of neutrophils, macrophages, and eosinophils as well as in activation of intramural mast cells.

When comparing levels of LTE4 and 9a11bPGF2 in exacerbated COPD and after allergen provocation in asthmatic patients, the role of these eicosanoids in developing bronchial obturation in COPD patients seems secondary. Some authors suggested administration of cysLT1receptor antagonists or cysteinyl leukotriene synthesis blockers in both stable and exacerbated COPD, but this seems insufficiently grounded. On the other hand, the presented study includes measurements of systemic levels of mediators, which are in fact synthesized and exert their action locally (in the respiratory tract). Further studies on bronchospastic eicosanoids, with analysis of their levels *in situ*, in lung parenchyma, and bronchial epithelium as well as in bronchioloalveolar lavage and induced sputum samples, can verify these observations.

Conclusions

Urine levels of LTE4 and 9a11bPGF2 in patients with exacerbated COPD do not significantly differ from concentrations found in healthy subjects. The mean concentrations of these eicosanoids in urine and levels of 9a11bPGF2 in serum during COPD exacerbation are similar to those found in stable disease. Patients with disease exacerbation had significantly increased urine LTE4 and serum 9a11bPGF2 levels when compared to healthy persons. These parameters also correlate with the number of cigarettes smoked by COPD patients.

The presented results suggest that eicosanoids play a minor role in the pathogenesis of bronchial obturation and COPD exacerbation, and their increasing levels in exacerbation periods reflect

induced inflammatory reaction, with increased influx of inflammatory cells to bronchial mucosa and lung parenchyma.

Conflict of interests

The authors declare no conflicts of interest.

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