ORIGINAL ARTICLE

The Relationship Between Oxidative Stress and Acid Stress in Adult Patients With Mild Asthma

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Abstract

Background: Oxidative stress plays an important role in the pathogenesis of asthma. Interestingly, a low airway pH and a high concentration of 8-isoprostane, a marker of oxidative stress, has been reported to cause inflammatory airway diseases. However, the relationship between these 2 markers and pulmonary function has not been determined in mild asthma patients.
Methods: pH and 8-isoprostane concentration were measured in exhaled breath condensate (EBC) from patients with mild asthma (n = 44) and healthy subjects (n = 20). The relationship between acid stress (pH) and oxidative stress (8-isoprostane) was then analyzed, along with the relationships between these 2 markers and lung function.
Results: The median (interquartile range [IQR]) pH of EBC was significantly lower in asthma patients than in control subjects (7.53 [7.41-7.68] vs 7.70 [7.62-7.74], $P < .05$), while the median (IQR) 8-isoprostane concentration of EBC was significantly higher in asthma patients than control subjects (16.2 [11.7-19.1] vs 3.5 [2.6-7.9] pg/mL, $P < .05$). There was no correlation between pH and 8-isoprostane concentration. Furthermore, lung function was not correlated with either pH or 8-isoprostane concentrations in EBC.
Conclusions: Acid stress and oxidative stress assessed by pH and 8-isoprostane concentration, respectively, in EBC did not show parallel changes associated with asthma and were not correlated with lung function in asthma patients. These 2 stress factors may have different roles in the pathogenesis of asthma.

Key words: Asthma. Oxidative stress. Acid stress. Exhaled breath condensate.

Resumen

Antecedentes: El estrés oxidativo tiene una función muy importante en la patogenia del asma. Una observación interesante, ha determinado que un pH bajo de las vías respiratorias y una elevada concentración de 8-isoprostano, un marcador del estrés oxidativo, provocan enfermedades inflamatorias de las vías respiratorias. No obstante, no se ha determinado la relación entre estos dos marcadores y la función respiratoria en pacientes con asma leve.
Métodos: Se midieron el pH y la concentración de 8-isoprostano en un condensado de aire espirado (CAE), en pacientes con asma leve (n = 44) y en sujetos sanos (n = 20). La relación entre el estrés ácido (pH) y el estrés oxidativo (8-isoprostano) se analizó, entonces, junto con las relaciones entre estos 2 marcadores y la función respiratoria.
Resultados: La media (rango intercuartílico [RIC]) de pH del CAE fue significativamente más baja en pacientes con asma que en los sujetos control (7,53 [7,41-7,68] frente a 7,70 ([7,62-7,74], $P < 0,05$), mientras que la media (RIC) de concentración de 8-isoprostano del CAE fue significativamente mayor en los pacientes con asma que en los sujetos control (16,2 [11,7-19,1] frente a 3,5 [2,6-7,9] pg/mL, $P < 0,05$). No hubo ninguna correlación entre el pH y la concentración de 8-isoprostano. Además, la función respiratoria no se correlacionó ni con el pH, ni con las concentraciones de 8-isoprostano en el CAE.
Conclusiones: El estrés ácido y el estrés oxidativo evaluados mediante el pH y la concentración de 8-isoprostano, respectivamente, en el CAE no mostraron cambios paralelos asociados con el asma y no estaban correlacionados con la función respiratoria en los pacientes con asma. Estos dos factores de estrés pueden tener diferentes funciones en la patogenia del asma.

Palabras clave: Asma. Estrés oxidativo. Estrés ácido. Condensado de aire espirado.

Introduction

Asthma is a condition involving chronic airway inflammation and oxidative stress [1]. Oxidant generation is part of the normal metabolism of many types of cells and is critical for cell homeostasis. To protect itself against exposure to noxious oxidants, the lung has a well-developed antioxidant system. When an imbalance occurs between oxidants and antioxidants in favor of oxidants, oxidative stress is said to occur. Many experimental and clinical findings suggest that oxidants play a role in the pathogenesis of several respiratory disorders, including bronchial asthma. In particular, there is increasing evidence that the chronic airway inflammation typical of asthma results in increased oxidative stress in the airways, as indicated by elevated levels of oxidative products in asthma patients [2]. F_2-isoprostanes are considered to be the most important markers of oxidative stress [3]. Because they are synthesized from arachidonic acid by free radical-catalyzed lipid peroxidation, their synthesis is mainly independent of cyclooxygenase [4]. Among the F_2-isoprostanes, 8-isoprostane has been the most extensively studied, and increased 8-isoprostane levels have been detected in plasma, urine, and bronchoalveolar lavage (BAL) fluid from asthma patients [4].

Acidification of the fluid lining the airways can affect airway function through numerous mechanisms. A decrease in the pH of the airways has been reported to cause bronchoconstriction [5], impair ciliary motility [6], increase the viscosity of airway mucus [7], and damage the airway epithelium [8], and these changes are crucial for the development of inflammatory airway diseases such as asthma [5-8]. Acidification is a common finding in fluids associated with inflammation throughout the body, and it is reasonable to expect the same in the lung in asthma and other inflammatory airway diseases [5,8]. This possibility has aroused interest in the effects of acid stress on the airways of asthmatics, and it has been demonstrated that, in addition to oxidative stress, acid stress also has an important role in the pathophysiology of asthma [5]. However, there have been no studies that have directly assessed the relationship between acid stress and oxidative stress in asthma patients.

In the present study, pH levels were compared in exhaled breath condensate (EBC) from adult asthma patients and healthy control subjects. In addition, the relationship between the pH of EBC and the concentration in EBC of a marker of oxidative stress, 8-isoprostane, was investigated to determine whether there was a close connection between acid stress and oxidative stress. The relationship between these markers and lung function was also studied.

Methods

Subjects

Adults aged 18 years or over with asthma were recruited from among outpatients of Gunma University Hospital (Table). The diagnosis of asthma was made as described previously [9], and the severity of asthma was classified according to National Institutes of Health–World Health Organization guidelines [10].

Table. Demographic and Clinical Variables of the Study Group[a]

	Control Subjects	Asthma Patients
No.	20	44
Age, y	35.2 (2.8)	37.6 (1.5)
Sex, F/M	8/12	20/24
FEV_1, % predicted	112 (5.4)	90 (4.8)

Abbreviations: F, female; M. male; FEV_1, forced expiratory volume in 1 second.
[a]Data are shown as means (SEM) unless otherwise indicated.

Briefly, mild asthma was defined as symptoms occurring no more than twice a week, with a forced expiratory volume in 1 second (FEV_1) ≥ 80% of the predicted value, reversibility of more than 15% with salbutamol, or a histamine provocation concentration causing a 20% fall in FEV_1 of less than 8 mg/mL histamine. All of the asthma patients were nonsmokers. In addition, sex-matched and age-matched healthy volunteers without respiratory disease were recruited as control subjects. They were all nonsmokers and had no history of allergy, as well as having no known respiratory diseases such as asthma, chronic obstructive pulmonary disease, or bronchiectasis. They had no chronic respiratory symptoms and had been free from respiratory tract infection for at least 6 weeks prior to assessment. The Ethics Committee of Gunma University approved the study and informed consent was obtained from all of the subjects.

Lung Function

Spirometry was carried out with a CHESTAC-55V spirometer (CHEST MI, Tokyo, Japan), and the best value from 2 maneuvers was recorded as an absolute value and as a percentage of the predicted value of FEV_1.

Collection of EBC

EBC was collected using an EcoScreen condenser (Jaeger, Berlin, Germany) according to the manufacturer's instructions. Collection was performed from 09:00 to 10:00 in the morning. The subjects were not allowed to eat or drink for at least 2 hours before EBC collection. After rinsing the mouth, tidal breathing was continued through a mouthpiece that was connected via a 1-way valve to a cooled collection tube in which vapor, aerosol, and moisture from the breath condensed on the walls. The design of the system prevented any contamination of EBC by saliva. Each subject was asked to breathe into the device for 10 minutes while wearing a nose clip. Using this method, approximately 2 mL of breath condensate was collected and was divided in 2 equal samples in 2-mL sterile plastic tubes. The first sample was used for the measurement of pH, while the second was immediately frozen and stored at –70°C for no longer than 2 months prior to measurement of 8-isoprostane (samples were thawed immediately prior to analysis). To rule out contamination of EBC by saliva, the amylase concentration of samples was measured as described elsewhere [11]. Amylase was undetectable in all of the samples tested.

Measurement of pH

A stable pH was achieved in all cases immediately after deaeration and decarbonation of the EBC specimens by gentle bubbling with nitrogen for 10 minutes, as described previously [12]. The pH was measured with a Cardy Twin pH meter (Horiba, Kyoto, Japan), with a range of 0.00 to 14.00 and a mean resolution/accuracy in the order of 0.01 ± 0.02 pH units. Measurement was initially done at 5, 10, and 15 minutes after deaeration/decarbonation with nitrogen. Because we observed no difference between the 2 last measurements, we assumed that the time necessary to obtain free carbon dioxide samples was 10 minutes. In order to avoid contamination with ambient CO_2 that could influence pH values, the EBC vials were tightly capped during the pH measurement.

Measurement of 8-Isoprostane

The concentration of 8-isoprostane in EBC was measured using a specific enzyme immunoassay kit (Cayman Chemical, Ann Arbor, USA), as described elsewhere [13]. Intra-assay and interassay variation was 5% and 6%, respectively, and the detection limit of the assay was 4 pg/mL.

Repeatability of pH and 8-Isoprostane Measurements

The within-subject repeatability of pH and 8-isoprostane measurements was assessed by collection of EBC as described above at the same time of day (09:00-10:00) for 6 consecutive days in healthy volunteers. They were all nonsmoking subjects who had no known respiratory disease, who reported no symptoms of respiratory tract infection, and who had not taken medications for at least 4 weeks prior to the study.

Statistical Analysis

Data were analysed with the Statistical Package for the Social Sciences (SPSS) for Windows version 11.5 (SPSS Inc, Chicago, IL, USA). Demographic data on the subjects were presented as the mean (SEM). The pH and 8-isoprostane levels were expressed as the median and interquartile range (IQR) and were compared between the asthma patients and control subjects using the Kruskal-Wallis test or the Mann-Whitney rank-sum test, as appropriate. Correlations between variables were assessed by using the Spearman rank correlation test. The repeatability of pH and 8-isoprostane measurements was assessed by calculating the coefficient of variation. A value of $P < .05$ was considered to indicate statistical significance.

Results

A total of 44 adults with mild asthma and 20 age- and sex-matched healthy controls were recruited for the study. The demographic and clinical characteristics of the subjects are summarized in the table. In all of the subjects, sufficient EBC was obtained for measurement. There were no significant differences in the median (IQR) volume of EBC obtained between the mild asthma patients (1.8 mL [1.5-2.1]) and the control subjects (1.6 mL [1.1-1.8]). The median (IQR) pH of EBC was significantly lower in the patients with mild asthma

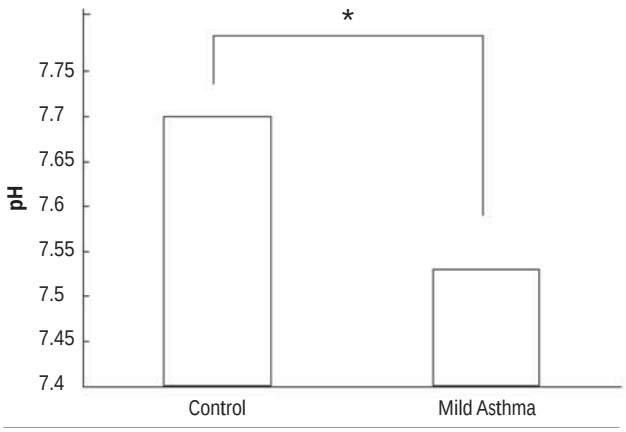

Figure 1. Mean pH in exhaled breath condensate from patients with mild asthma and control subjects. *$P = .0007$.

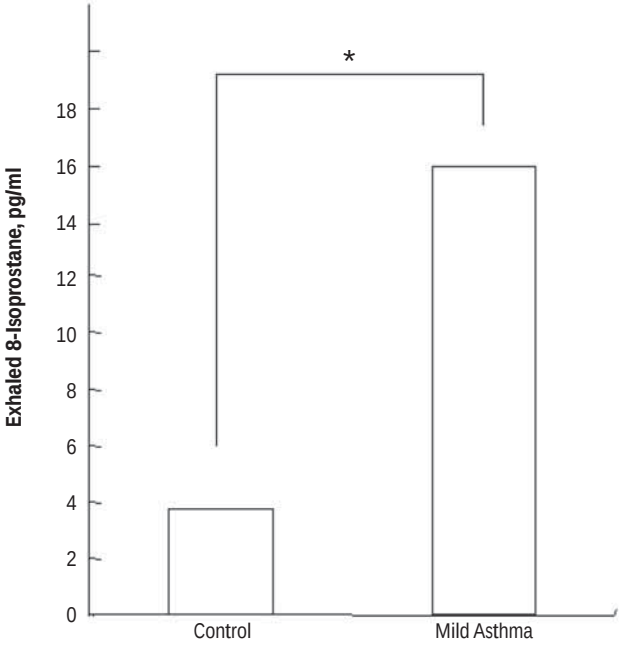

Figure 2. Mean 8-isoprostane concentrations in exhaled breath condensate from asthma patients and control subjects. *$P = .0335$.

than in the control subjects (7.53 [7.41-7.68] vs 7.70 [7.62-7.74], $P < .05$). The mean pH of EBC from the asthma patients and control subjects is shown in Figure 1. The concentration of 8-isoprostane was detectable in EBC from all subjects, and the median (IQR) concentration of 8-isoprostane was significantly higher in the asthma patients than in the healthy control subjects (16.2 [11.7–19.1] vs 3.5 [2.6–7.9] pg/mL, $P < .05$). The mean 8-isoprostane concentrations in EBC from the asthma patients and control subjects are shown in Figure 2. No correlation was observed between the pH and the 8-isoprostane concentration in EBC (=–0.09, $P = .7$). Pulmonary function also showed no correlation with either 8-isoprostane concentration (=–0.07, $P = .79$) or the pH (= 0.16, $P = .42$) of EBC.

To assess the repeatability of measuring the pH and the 8-isoprostane levels in EBC, samples were collected on 6 consecutive days from 8 healthy adults aged 24 to 39 years. The mean coefficient of variation for measurements of the exhaled pH and 8-isoprostane levels was 4.8% (range, 2.6% – 7.7%) and 5.3% (range, 2.1% – 6.9%), respectively.

Discussion

In this study, we found that the pH of EBC was significantly lower in patients with mild asthma than in healthy control subjects, whereas the 8-isoprostane concentration of EBC was significantly higher in the asthma patients. There was no correlation observed between the pH and the 8-isoprostane level in EBC. In addition, pulmonary function did not show any correlation with either the exhaled 8-isoprostane concentration or the pH of EBC.

8-Isoprostane is the best characterized of the F_2 isoprostanes and has been shown to provide a reliable index of lipid peroxidation and oxidative stress in a variety of clinical settings [14]. Isoprostanes have various important biological effects in the airway and pulmonary vascular smooth muscle, as well as the airway epithelium, endothelium, and sensory nerves. Their actions may partly account for the nonspecific smooth muscle hyperreactivity, bronchoconstriction, and edema that characteristically occur in many lung diseases [14]. Increased systemic and airway levels of 8-isoprostane have been found in asthma patients [3,4]. Montuschi et al [13] recently demonstrated that the 8-isoprostane concentration in EBC was higher in adult asthma patients than in healthy control subjects, and was also higher in subjects with severe asthma than in those with milder disease. In agreement with their findings, our study showed that adults with mild asthma had a marked increase of 8-isoprostane in EBC compared with the level in EBC from healthy control subjects. This confirms that airway inflammation is associated with the increased production of reactive oxygen species in the lungs and supports a central role of oxidative stress in asthmatic inflammation.

Subglottic air is saturated with water that can condense during exhalation [15]. In patients with asthma, EBC contains high concentrations of reactive oxygen species, which may reflect inflammatory changes in the lower respiratory tract [16,17]. In the present study, we found that the pH of EBC from adults with mild asthma was significantly lower than that of EBC from a control group. These results support an important role of acid stress in the pathophysiology of asthma and confirm the findings of Hunt et al [16] and Kostikas et al [18], who reported that the pH of EBC from adults with mild asthma was significantly lower than that of EBC from a control group. However, our data disagree with the findings of Nicolaou et al [19] that the pH of EBC from adults with mild asthma was not significantly lower than that of EBC from a control group.

It is interesting to note that the interactions and toxicity of many reactive oxygen species are critically pH dependent. In rats, experimental acidification of the fluid lining the airways increases the local production of reactive oxygen species [20]. In addition, an acidic pH greatly augments oxidative damage caused by hyperoxia in rats, partly because of the decline of antioxidant capacity [21], and we have found that gastroesophageal reflux disease is common in asthma patients [22]. Therefore, we hypothesized that there might be a close relationship between oxidative stress and acid stress (assessed from the 8-isoprostane level and pH of EBC, respectively) in asthma patients. However, we did not observe any such correlation between the 8-isoprostane concentration and the pH of EBC in the present study. Our findings suggest that acid stress in the lungs might not be parallel and complementary to oxidative stress, but that these 2 mechanisms may have different roles in the pathogenesis of asthma. Moreover, we did not find any correlation between spirometric parameters and the pH or 8-isoprostane level in EBC. This suggests that the pH and 8-isoprostane level in EBC may reflect the intensity of current inflammation, whereas lung function tests are at least partly influenced by long-standing pulmonary damage. Thus, these analyses may complement each other when assessing the severity of asthma.

Analysis of EBC is a novel tool that has been developed in recent years, and it is likely to reflect the local production of free radicals rather than systemic production [23]. Although no correlation was reported in comparisons of biomarkers from BAL and EBC [24], the noninvasive nature of EBC offers some advantages over invasive techniques such as BAL, bronchial biopsy, and sputum induction. Because EBC can be collected noninvasively, repeated measurements can be made during follow-up of patients. In addition, this tests can be performed in patients with poor pulmonary function in whom bronchoscopy and sputum induction may be too hazardous. However, standardization of the method and validation of the results of analysis are needed before EBC studies can be used as a clinical tool [25-28].

In conclusion, we found that both oxidative stress and acid stress were associated with the pathophysiology of asthma, as reflected by the 8-isoprostane level and pH of EBC. Investigation of acid stress is a new area of research with potential applications for the monitoring of asthma and for development of new therapies. However, we did not observe any correlation between the 8-isoprostane level and pH of EBC. Our findings suggest that acid stress in the lungs might not be parallel and complementary to oxidative stress, but that these may represent 2 different mechanisms contributing to the pathogenesis of asthma.

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