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H. 知的財産権の出願・登録状況

(予定を含む。)

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

特記なし

Ⅱ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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Ⅲ. 研究成果の刊行物

INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Peak Expiratory Flow Variability Adjusted by Forced Expiratory Volume in One Second is a Good Index for Airway Responsiveness in Asthmatics

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Abstract

Background The lowest peak expiratory flow (PEF) over a week, expressed as a percentage of the highest PEF (Min%Max PEF) has been reported to be the index that most closely correlates with airway hyperresponsiveness (AHR) in asthmatics. However, both fluctuation of the airway caliber and airflow limitation are regarded as physiological properties of asthma closely related to AHR. An accurate index that shows the degree of AHR may be obtained by combining the index of airway lability with the parameters that represent airway caliber.

Methods Ninety-two steroid-naive and twenty-eight steroid-treated asthmatic patients were enrolled. Using the physiological parameters obtained from spirometry and PEF monitoring, we investigated the indices which correlate accurately with airway responsiveness measured by the inhalation challenge test.

Results Although the methacholine threshold was related to all parameters that represent airway caliber and lability, Min%Max PEF had the strongest correlation with AHR. When Min%Max PEF was adjusted by the airway geometric factors, the normalization of Min%Max PEF with forced expiratory volume in one second as a percentage of the predicted value (%FEV_1) improved the relationship between Min%Max PEF and AHR.

Conclusions Min%Max PEF adjusted by %FEV_1 showed a good correlation with airway responsiveness measured by the inhalation challenge test, and may be useful as a convenient alternative index of AHR in asthmatic patients

Key words: airway hyperresponsiveness, airway lability, airflow limitation, bronchial asthma, spirometry

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Introduction

Airway hyperresponsiveness (AHR), the degree of airway responsiveness to various nonspecific stimuli, is an important physiological feature of asthma (1-3). It has been reported that evaluation of AHR is useful to diagnose asthma (1, 2), assess the response to asthma therapy (4-6), and guide asthma treatment (7, 8). However, the standard method of assessing AHR, inhalation challenge test, is not easy to perform in clinical practice.

Although multiple factors are involved in the mechanism of AHR, both fluctuation of the airway caliber and airflow limitation are regarded as physiological properties of asthma closely related to AHR (3, 4, 9-14). Peak expiratory flow (PEF) monitoring is accepted as a part of asthma management that provides information about fluctuations of the airway caliber, known as airway lability (1). Among several PEF indices, the lowest PEF over a week, expressed as a percentage of the highest PEF (Min%Max PEF) has been suggested to be the best index of airway lability in clinical practice because it more strongly correlates with AHR than

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Table 1. Subject Demographics

	Steroid-naive	Steroid-treated
Number	92 (F/M = 57/35)	28 (F/M = 15/13)
Asthma status	Mild persistent = 58	Controlled = 25
	Moderate persistent = 34	Pertly controlled = 3
Age (Years)	45.7±1.7	41.1±1.9
FVC (L)	3.28±0.08	3.56±0.14
FEV_1 (L)	2.54±0.08	2.85±0.11
FEV_1% (%)	77.2±0.1	80.5±1.6
%FEV_1 (%)	91.0±1.2	92.4±1.7
Rrs (cmH_2O/L/s)	4.3±0.1	4.6±0.3
Min PEF (L/min)	342±12	379±21
Max PEF (L/min)	410±12	442±22
Mean PEF (L/min)	376±12	410±22
Min%Max PEF (%)	82.8±0.8	85.3±1.1
PD_{200} (mg/mL)	14.0±2.1	16.6±1.7

Definition of abbreviations: F: female, M: male, FVC: forced vital capacity, FEV_1: forced expiratory volume in one second, %FEV_1: FEV_1 expressed as a percentage of the predicted value, Rrs: respiratory resistance, PEF: peak expiratory flow, Min PEF: the lowest PEF over a week, Max PEF: the highest PEF over a week, Mean PEF: the mean value of the lowest and highest PEF, Min%Max PEF: the lowest PEF over a week, expressed as the percentage of the highest PEF, PD_{200}: cumulative provocative dose of methacholine causing a 100% increase in respiratory resistance. Values are mean±SE.

any other physiological parameters (12). However, a close association was also found between lower level parameters that represent airway caliber such as forced expiratory volume in one second (FEV_1) and AHR (3, 4, 9, 10). Therefore, we hypothesized that an accurate index that shows the degree of AHR may be obtained by combining the index of airway lability with the parameters that represent airway caliber.

In the current study, using the physiological parameters obtained from spirometry and PEF monitoring, we investigated the indices which correlate accurately with AHR measured by the inhalation challenge test in patients with asthma.

Methods

Study subjects

Ninety-two steroid-naive and twenty-eight steroid-treated, nonsmoking asthmatic patients took part in the study after giving informed consent. The study was approved by the local ethics committee. All patients satisfied the American Thoracic Society criteria for asthma (15). The clinical characteristics of the study subjects are shown in Table 1. All patients in the steroid-naive group attended our outpatient clinic recently, and had been without regular asthma treatment including steroid therapy. The asthma severity was classified in fifty-eight subjects as mildly persistent, and in thirty-four as moderately persistent (1). The patients in the steroid-treated group had been treated with inhaled corticosteroids at a mean equivalent dose of 372 μg fluticasone propionate·day^{-1} without any other regular asthma treatment. The asthma control levels of twenty-five steroid-treated patients were classified as controlled, but three patients were classified as partly controlled because their FEV_1 value were below 80% of the predicted values (1). Rescue use of short acting inhaled β_2 agonists as needed for relief of symptoms was permitted. Because of safety concerns with methacholine challenge testing, steroid-naive subjects whose asthma severity was classified as severe persistent, steroid-treated patients whose control level was classified as uncontrolled, and subjects with impaired lung function (FEV_1< 55% predicted value) were not enrolled in this study. In addition, subjects were not included if they had had an exacerbation of asthma, or a respiratory tract infection, in the two weeks preceding the study, or the use of rescue inhaled β_2 agonists within twenty-four hours before the inhalation challenge test.

Study design

The present study was cross-sectional. Subjects attended the outpatient clinic at the Wakayama Medical University hospital on one occasion for clinic examination, spirometry, and methacholine inhalation challenge. PEF monitoring was performed for at least two weeks before this attendance.

Methacholine inhalation challenge test

Airway responsiveness was measured using a device (Astograph Jupiter 21; Chest Co., Tokyo, Japan) that displays respiratory resistance (Rrs) measured via the forced oscillation method during tidal breathing with continuous inhalation of the aerosolized methacholine as previously described (16). The degree of the airway responsiveness was defined as the cumulative provocative dose of methacholine causing

Table 2. Correlation between Airway Responsiveness to Methacholine and Airway Physiologic Parameters

Physiologic parameters	Coefficient	p value	95% CI
Rrs ($cmH_2O/L/s$)	-0.461	< 0.0001	-0.591 , -0.307
FVC (L)	0.287	< 0.005	0.113 , 0.443
FEV_1 (L)	0.408	< 0.0001	0.246 , 0.547
$\%FEV_1$ (%)	0.607	< 0.0001	0.480 , 0.709
Min PEF (L/min)	0.449	< 0.0001	0.293 , 0.581
Max PEF (L/min)	0.297	< 0.001	0.124 , 0.452
Mean PEF (L/min)	0.374	< 0.0001	0.209 , 0.519
Min%Max PEF (%)	0.709	< 0.0001	0.607 , 0.788

Definition of abbreviations: CI: confidence interval, Rrs: respiratory resistance, FVC: forced vital capacity, FEV_1: forced expiratory volume in one second, $\%FEV_1$: FEV_1 expressed as a percentage of the predicted value, PEF: peak expiratory flow, Min PEF: the lowest PEF over a week, Max PEF: the highest PEF over a week, Mean PEF: the mean value of the lowest and highest PEF, Min%Max PEF: the lowest PEF over a week, expressed as the percentage of the highest PEF

a 100% increase in baseline Rrs (PD_{200}) (17).

Pulmonary function test

FEV_1 and forced vital capacity (FVC) were measured with a Chest HI 801 (Chest Co., Tokyo, Japan) according to the standard procedure (18).

PEF measurements

Using an Assess® peak flow meter (Respironics Health Scan Inc., Cedar Grove, NJ, USA), PEF measurements were performed twice a day for at least two weeks according to the standard procedure (19). The lowest PEF expressed as a percentage of the highest PEF in the previous week before the methacholine inhalation challenge test was assumed to represent the PEF variability for the week (Min%Max PEF) (1, 12).

Adjustment of PEF variability for physiological parameters that represent airway caliber

To investigate the physiological indices which correlate accurately with AHR in asthmatics, Min%Max PEF was adjusted by several airway geometric factors. Seven separate indices were calculated as follows:

1. Min%Max PEF adjusted by Rrs

The Min%Max PEF was divided by the actual Rrs value.

2. Min%Max PEF adjusted by FVC

The Min%Max PEF was multiplied by the actual FVC value.

3. Min%Max PEF adjusted by FEV_1

The Min%Max PEF was multiplied by the actual FEV_1 value.

4. Min%Max PEF adjusted by $\%FEV_1$

The Min%Max PEF was multiplied by the FEV_1 percentage of the predicted value ($\%FEV_1$).

5. Min%Max PEF adjusted by Minimum PEF

The Min%Max PEF was multiplied by the actual lowest PEF value during one week.

6. Min%Max PEF adjusted by Maximal PEF

The Min%Max PEF was multiplied by the actual highest PEF value during one week.

7. Min%Max PEF adjusted by Maximal PEF

The Min%Max PEF was multiplied by the actual mean value of the lowest and highest PEF during one week.

Statistical analysis

Spearman's correlation coefficients were calculated to determine the correlation between the methacholine threshold and pulmonary physiological parameters. All data were expressed as means ± SE, and significance was defined as a p value of less than 0.05.

Results

Correlation between airway physiological parameters and airway responsiveness

The results of the correlation coefficient analysis between the airway responsiveness and airway physiologic parameters are listed in Table 2. Although the PD_{200} was related to all parameters that represent airway caliber and lability (Table 2 and Fig. 1), Min%Max PEF had the strongest correlation with AHR ($r=0.709$, $p<0.0001$) (Table 2 and Fig. 2A).

Impact of adjusting the PEF variability for airway caliber on the correlation with AHR

When Min%Max PEF was adjusted for airway geometric factors, the normalization with $\%FEV_1$ improved the correlation between Min%Max PEF and AHR ($r=0.750$, $p<0.0001$) (Table 3 and Fig. 2B). By contrast, other geometric factors such as actual FEV_1 and Rrs values did not improve the relationship between Min%Max PEF and airway responsiveness (Table 3). A nomogram incorporating Min%MaxPEF with $\%FEV_1$ was constructed to predict the degree of airway responsiveness measured by the methacholine challenge test (Fig. 3). The PD_{200} value was calculated by the correlation equation obtained from regression analysis, as follows: $\log PD_{200}=\text{Min\%Max PEF}\times\%FEV_1/1885-2.9$.

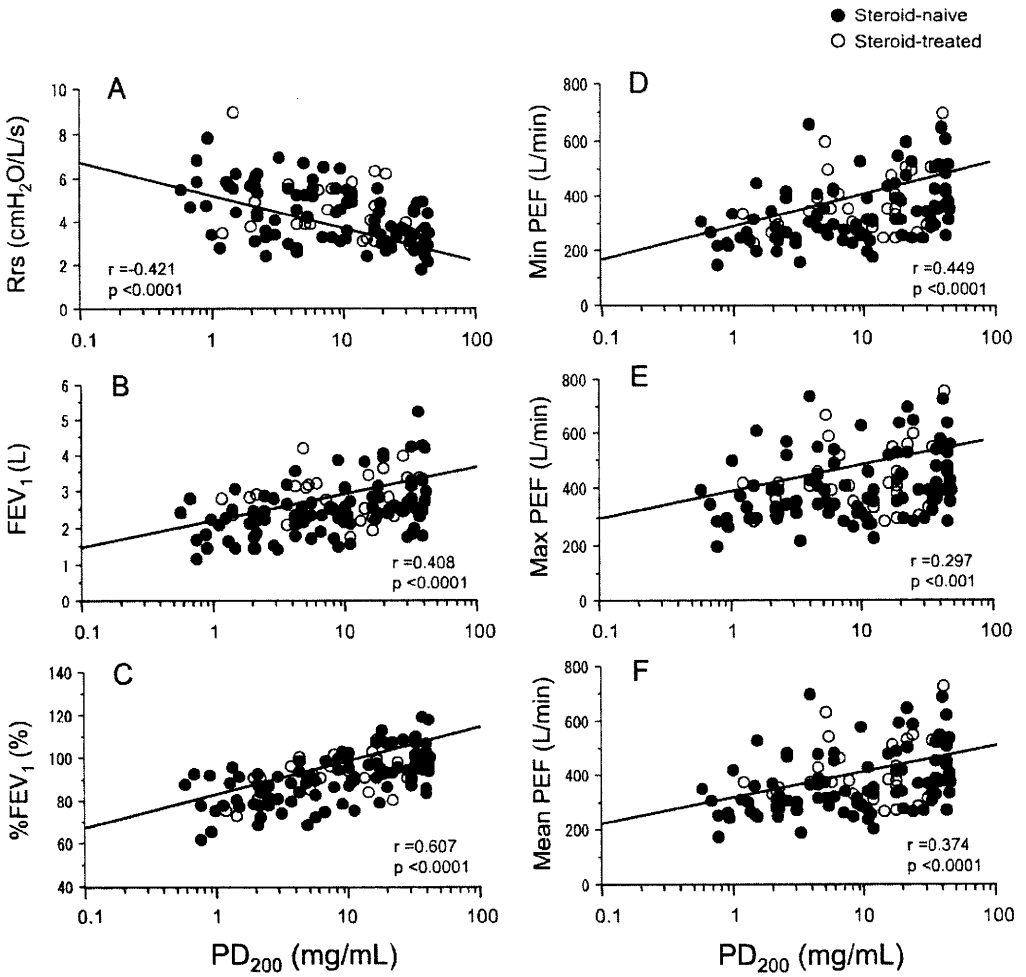

Figure 1. Relationship between airway responsiveness to methacholine and physiological parameters that represent airway caliber: a) respiratory resistance value; b) actual forced volume in one second (FEV_1) value; c) FEV_1 percentage of the predicted value ($\%FEV_1$); d) the lowest peak expiratory flow (PEF) value during one week; e) the highest PEF value during one week; f) mean value of the lowest and highest PEF value. The lines correspond to the fitted regression equation.

Discussion

In the current study, the correlation coefficient analysis indicated that airway responsiveness measured by the inhalation challenge test correlated with the parameters that represent airway caliber and lability, and Min%Max PEF had the strongest correlation with AHR in asthmatics. However, the normalization of Min%Max PEF with $\%FEV_1$ improved the relationship between Min%Max PEF and AHR.

AHR is a consistent and defining feature of asthma (1-3). AHR measurement is a valuable tool in the diagnosis of asthma (1, 2), and for evaluating the treatment response (4-6). In addition, it has been demonstrated that asthma management plans that include AHR measurements are superior to plans without AHR measurements (7, 8). Although airway responsiveness is generally evaluated by inhalation challenge test using bronchoconstrictive agents, it is not convenient and involves several clinical issues such as invasiveness and contraindications (2). Therefore, establishing a convenient index for predicting the degree of airway responsiveness other than by using the inhalation challenge test would be useful for clinical asthma management.

Several mechanisms, such as airway inflammation, increased neural reflexes, airway geometric factors, and genetic factors, have been proposed to explain the AHR (1-4). Among these mechanisms, airway inflammation has been reported to be a key factor (3, 4, 6, 7), and it also affects the other important physiologic properties of asthma, such as airflow limitation and airway lability (3, 4, 6). Previous studies have shown close correlations between PEF variability and AHR (11-14). In particular, Reddel et al recommended Min%Max PEF as the best index of airway lability in clinical practice (12). However, a reduction in airway caliber would result in a greater increase in airway resistance and consequently greater airflow limitation (20), and a close association was found between lower level parameters that represent airway caliber such as FEV_1 and AHR (3, 4,

Table 3. Correlation between Airway Responsiveness to Methacholine and Indices that Variability of Peak Expiratory Flow Adjusted by Airway Geometric Factors

Adjusting factors	Coefficient	p value	95% CI
Not adjusted	0.709	< 0.0001	0.607 , 0.788
Rrs (cmH_2O/L/s)	0.577	< 0.0001	0.444 , 0.686
FVC (L)	0.473	< 0.0001	0.322 , 0.602
FEV_1 (L)	0.532	< 0.0001	0.390 , 0.649
$\%FEV_1$ (%)	0.750	< 0.0001	0.659 , 0.819
Min PEF (L/min)	0.538	< 0.0001	0.398 , 0.654
Max PEF (L/min)	0.449	< 0.0001	0.293 , 0.581
Mean PEF (L/min)	0.495	< 0.0001	0.347 , 0.619

Definition of abbreviations: CI: confidence interval, Rrs: respiratory resistance, FVC: forced vital capacity, FEV_1: forced expiratory volume in one second, $\%FEV_1$: FEV_1 expressed as a percentage of the predicted value, PEF: peak expiratory flow, Min PEF: the lowest PEF over a week, Max PEF: the highest PEF over a week, Mean PEF: the mean value of the lowest and highest PEF, Min%Max PEF: the lowest PEF over a week, expressed as the percentage of the highest PEF

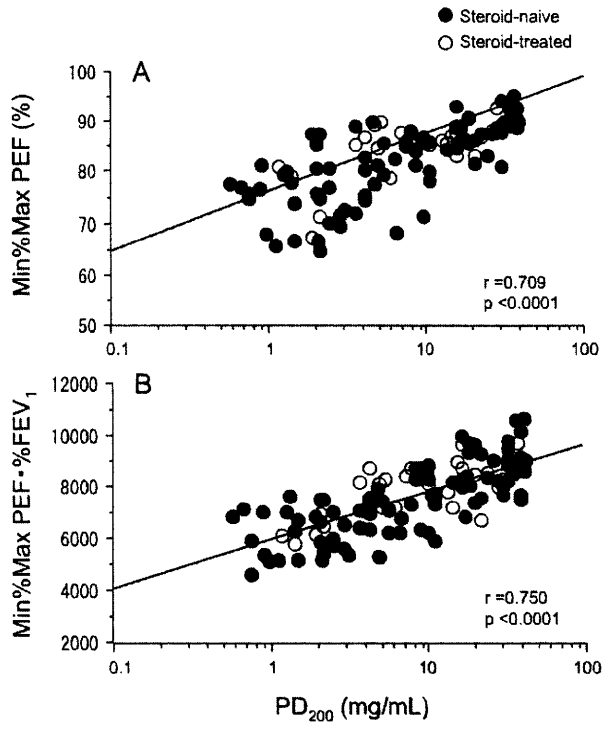

Figure 2. Relationship between airway responsiveness and the lowest peak expiratory flow (PEF) during one week expressed as a percentage of the highest PEF (Min%Max PEF) normalized with or without forced volume in one second (FEV_1) percentage of the predicted value ($\%FEV_1$): a) Min%Max PEF; b) Min%Max PEF adjusted by $\%FEV_1$. The lines correspond to the fitted regression equation.

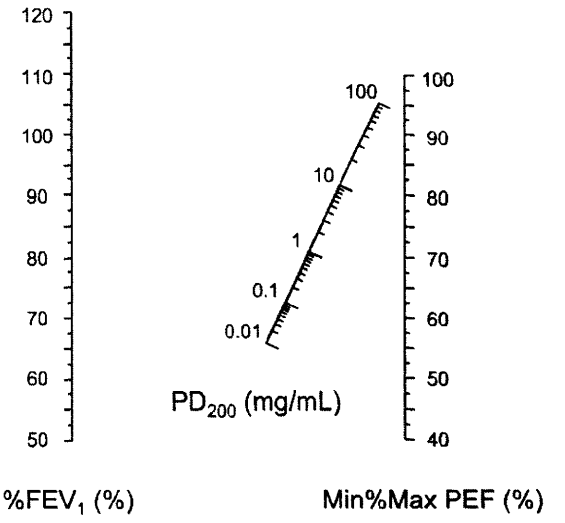

Figure 3. The nomogram to predict the degree of airway responsiveness in asthmatic subjects incorporating the variability of peak expiratory flow (Min%Max PEF) and forced expiratory volume in one second percentage of the predicted value ($\%FEV_1$). A cumulative provocative dose of methacholine causing a 100% increase in respiratory resistance (PD_{200}) was calculated by a correlation equation obtained from the regression analysis, as follows: $\log PD_{200}$=Min%Max PEF× $\%FEV_1$/1885-2.9.

9, 10). Therefore, we sought to investigate the indices which correlate accurately with AHR by combining the index of airway lability with the parameters that represent airway caliber.

In fact, the current study demonstrated that $\%FEV_1$ was the airway geometric factor that correlated well with AHR, and adjusting Min%Max PEF for $\%FEV_1$ improved the correlation between Min%Max PEF and AHR. Airflow limitation in subjects with asthma may be reversible or fixed, and appears to represent a different dimension of asthma severity from airway lability. Several previous studies have shown that airflow limitation can be present with normal PEF variability and severe AHR (21-24). In addition, PEF measurements can underestimate the degree of airflow limitation, particularly as airflow limitation and gas trapping worsen (1). These are possible explanations for the fact that adjust-

ing the PEF variability for $\%FEV_1$ improved the relationship between Min%Max PEF and AHR. Actually, the improvement of this relationship by adjusting it by $\%FEV_1$ seemed to be more remarkable in the cases with higher airway responsiveness. By contrast, other geometric factors such as actual FEV_1 and Rrs values had significant but weak correlations with the methacholine threshold, and consequently the normalization of Min%Max PEF with these geometric factors did not improve the relationship between Min%Max PEF and AHR.

Min%Max PEF adjusted by $\%FEV_1$ appeared to be a convenient alternative to the index of AHR in asthmatic patients, and the resulting nomogram that could predict the degree of airway responsiveness may be useful for clinical asthma management. Because this proposed index correlated accurately with the degree of AHR, and it could be obtained by using conventional airway physiological parameters measured by spirometry and PEF monitoring. However, precise instruction is necessary so that patients can reliably measure PEF (1). In addition, although it has been demonstrated that PEF variability is the most useful index for reflecting AHR longitudinally in treated asthmatic patients, its correlations are not very strong (13). Thus, a longitudinal validation study is needed to clarify the utility of adjusting the measurements of Min%Max PEF by $\%FEV_1$ as a clinical index for the changes in AHR by pharmacologic interventions for asthma.

In conclusion, Min%Max PEF adjusted by $\%FEV_1$ showed a good correlation with the airway responsiveness measured by the inhalation challenge test, and may serve as a convenient alternative to the index of AHR in asthmatic patients.

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Forum Review

Oxidative and Nitrative Stress in Bronchial Asthma

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ABSTRACT

There has been a marked increase in the global prevalence, morbidity, and mortality of asthma, and its associated economic burden has also grown over the last 40 years. Approximately 300 million people worldwide currently have asthma, and its prevalence increases by 50% every decade. Airway inflammation is the most proximate cause of the recurrent episodes of airflow limitation in asthma. Recent research has revealed that numerous biologically active proinflammatory mediators are responsible for the pathogenesis of asthma. Among these mediators, there is increasing evidence that endogenous or exogenous reactive oxygen species (ROS) and reactive nitrogen species (RNS) are responsible for the airway inflammation of asthma. Many reports have shown that there is an excessive production of ROS and RNS in the airways of asthmatic individuals compared with healthy subjects. Excessively produced ROS and RNS have been reported to lead to airway inflammation, airway hyper-responsiveness, airway microvascular hyperpermeability, tissue injury, and remodeling in animal models and human studies. Although human lungs have a potent antioxidant system, excessive oxidative and nitrative stress leads to an imbalance of oxidants/antioxidants. This review describes the rapidly accruing data linking oxidative and nitrative events to the pathogenesis of bronchial asthma. *Antioxid. Redox Signal.* 10, 785–797.

INTRODUCTION

In various inflammatory lung diseases, reactive oxygen species (ROS) and reactive nitrogen species (RNS), including superoxide anion, hydroxyl radicals, hydrogen peroxide, hypochlorous acid, ozone, and peroxynitrite, have been reported to play a pivotal role in the airway inflammation and pathogenesis (58, 84, 111). Among the inflammatory lung diseases, especially in bronchial asthma, oxidative and nitrative stress has been shown to be related to the pathogenesis (4, 9, 85). Oxidant generation is part of the normal metabolism of many types of cells and is critical for cell homeostasis. Although the lung has a well-developed antioxidant system to protect itself against exposure to endogenous or exogenous noxious oxidants (59, 86), excessively produced ROS and RNS can still cause inflammation in the lungs.

Asthma is a chronic inflammatory disease of the airways in which various resident and migrated cell-derived molecules play a role (20, 26). Many experimental and clinical data suggest that an imbalance between oxidants and antioxidants causes the airway inflammation and airway hyper-responsiveness that are major features of asthma (6, 13, 15). In animal models, allergen- (105) and ozone-induced (106) airway inflammation and airway hyper-responsiveness are largely modified by inhibitors of the synthesis of reactive oxygen and related species or by scavengers of radical species, supporting this hypothesis. Superoxide anion (O_2^-) may also be exaggerated in asthmatic airways via the upregulation of xanthine oxidase (XO) in microvascular endothelial cells (44) and NADPH oxidase in the infiltrated eosinophils (96). These results suggest that ROS may be related to the pathophysiology of bronchial asthma.

In addition, nitric oxide (NO) production is increased in asthmatic airways, possibly via the inducible type of NO synthase (iNOS) (33, 47), and steroid treatment reduces the NO generation (91). NO rapidly reacts with superoxide anion which is released from inflammatory cells, including eosinophils, re-

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sulting in the formation of the highly proinflammatory molecule, peroxynitrite (10, 82). In addition, since RNS, including peroxynitrite, cause tissue injury in a variety of organs, nitrative stress may be partly responsible for the airway inflammation in asthmatic patients.

This review describes the pathophysiological mechanisms and the clinical relevance of ROS and RNS in bronchial asthma. The role of ROS and RNS on airway remodeling observed in asthmatic airways is described. Furthermore, this review will examine the relationship between oxidative/nitrative stress and the refractoriness to steroids in refractory asthma.

ROS AND BRONCHIAL ASTHMA

Endogenous oxidants and bronchial asthma

In asthmatic airways, infiltrated inflammatory cells into the airways including eosinophils, neutrophils, and mast cells produce many oxidants by various stimuli (53, 76, 96). In particular, prominent eosinophil infiltration is observed in airways of asthmatic patients. Eosinophils are thought to injure the airway tissues by secreting proteins from its granules, including eosinophil cationic protein, major basic protein, and oxidants (24). It has been reported that eosinophils are stimulated by platelet activating factor (PAF) and injure epithelial cells in the presence of halogen atoms such as chloride and bromide (9). In addition, eosinophils, like neutrophils, have NADPH oxidase (24). In a previous study, eosinophils from asthmatic patients produced more superoxide anion than those from healthy subjects, suggesting that eosinophils from asthmatic patients may be primed by unknown stimuli (94). Moreover, macrophages (21) and neutrophils (25) from asthmatic patients are also reported to produce more oxidants than those from healthy subjects. In many animal asthmatic models, greater than normal amounts of endogenous ROS and RNS were produced (12, 17, 63, 87, 120). These results suggest that the production of ROS and RNS is upregulated in the airways of asthma, causing airway inflammation and hyper-responsiveness in bronchial asthma.

Exogenous oxidants and bronchial asthma

The lungs receive exogenous oxidative stress because of their exposure to the atmosphere. In a guinea pig model, exogenously administered ozone caused injury to epithelial cells, induced the infiltration of inflammatory cells into airway walls, and caused airway hyper-responsiveness (39), which resembled the pathophysiology of asthmatic airways. In this model, various possibilities were raised to explain the mechanism by which exogenously administered ozone induced the airway inflammation and hyper-responsiveness. First, ozone injures epithelial cells. Epithelial cells have neutral endopeptidase (NEP) and angiotensin converting enzyme (ACE) which catalyzes substance P (SP) and bradykinin (BK). SP and BK induce airway microvascular hyperpermeability (116) and smooth muscle contraction (109). The breakdown of NEP and ACE may prolong the survival of tachykinins, leading to the neurogenic inflammation observed in the airways of asthma. This hypothesis is supported by the finding that the levels are upregulated in the airways of asthmatic patients compared with healthy subjects (18, 19). In a guinea pig model of ozone-induced airway hyper-responsiveness, it was reported that ozone stimulated the release of histamine, prostaglandins, leukotrien B_4 (LTB_4), and thromboxane B_2 (TxB_2) (55). These mediators can induce bronchoconstriction and induce the infiltration of inflammatory cells into the airways (16). Cigarette smoke contains huge amounts of oxygen radicals and nitrogen species (83). It is a well-known fact that cigarette smoke worsens the airway inflammation and hyper-responsiveness in healthy subjects and asthmatic patients (74). The inhalation of exogenous oxidants may stimulate the inflammatory cells and worsen the airway inflammation in the airways of bronchial asthma.

Effect of oxidants on resident cells in lungs

Epithelial cells. Injury and shedding of epithelial cells are observed in the airways of asthmatic patients (26). These pathophysiological changes of epithelial cells are mediated by various noxious agents. Epithelial cells are exposed to exogenous oxidants and endogenous oxidants derived from infiltrated inflammatory cells, and are therefore thought to have the most frequent opportunities to receive oxidative stress. In fact, when eosinophils were stimulated by PAF, they injured epithelial cells *in vitro* (118). Since this type of injury was suppressed by catalase, hydrogen peroxide was responsible for this eosinophil-mediated epithelial cell injury (118). Once epithelial cells are injured, NEP and ACE are inactivated as described above. Furthermore, epithelial cells secrete much prostaglandin E2 (PGE_2) which has bronchodilatory action (36). The loss of epithelial cells by oxidative stress can stimulate airway smooth muscle cell contraction through the above-mentioned mechanisms. This epithelial cell injury leads to the loss of the barrier function. As a result of this loss, it is easy for various antigens and stimuli to achieve access to the airway tissue and thereby worsen the airway inflammation in asthmatic patients.

Airway smooth muscle cells. When 10^{-4}–10^{-3} *M* hydrogen peroxide is administered to the trachea of guinea pig, airway smooth muscle contraction is observed (88). This contraction is augmented when the epithelial cells are removed, suggesting that hydrogen peroxide-mediated airway smooth muscle cell contraction is related to the inactivation of relaxant factors such as prostaglandins derived from epithelial cells (36, 88). Since in asthmatic airways, there is shedding of epithelial cells, the contractile effect of endogenous or exogenous hydrogen peroxide in airway smooth muscle cells might be enhanced in asthmatic individuals.

Secretary cells. Inhalation of ozone induces excessive mucus secretion from secretary glands and goblet cells in the trachea of sheep (80). This hypersecretion is inhibited by cyclooxigenase (COX) inhibitors, suggesting that ozone could stimulate COX activity and the products of COX could be related to this excessive secretion. Furthermore, superoxide anion stimulates the production of mucin-like glycoproteins by airway epithelial cells through COX pathways (2). These results suggest that oxidants enhance mucus secretion in epithelial cells. The excessive secretion observed in the airways of asthmatic patients may be due to this oxidant-mediated COX activation.

Endothelial cells. Superoxide anion generated by the xanthine-xanthine oxidase system injures endothelial cells and enhances microvascular permeability *in vivo* (60). The amount of superoxide anion production shows a good correlation with the albumin in bronchoalveolar lavage fluid (BALf) from antigen-challenged asthmatic patients, suggesting that oxidants may cause the extravasation of serum in asthmatic airways (92). Recently, we showed that endogenous NO, superoxide anion, and peroxynitrite could augment the microvascular permeability during the late allergic response in guinea pigs (105). Each inhibitor or scavenger inhibited the microvascular hyperpermeability during the late allergic response (Fig. 1). In the airways of asthmatic patients, especially during exacerbations of bronchial asthma, marked edema of the airways was observed (48, 68, 69). Because excessive production of oxidants occurs in the airways of asthmatic patients during exacerbations, oxidants may be involved in the formation of airway wall edema.

Inflammatory cells. Oxidants are reported to have various effects on inflammatory cells. When inflammatory cells are exposed to oxidants, chemotactic factors are released from them through the arachidonic cascade (79). Moreover, when mast cells are exposed to oxidants, the release of histamine and serotonin is significantly increased (78). These mediators are related to the pathogenesis of bronchial asthma, and oxidants may be related to the inflammation of airways through this mechanism.

Other cells. ROS can oxidize the lipid membrane of many types of cells and activate the arachidonic cascade. The oxidation of the lipid membrane by oxidants can produce prostaglandins and leukotrienes (107). These products can contract airway smooth muscle cells, augment the chemotaxis of inflammatory cells toward inflammatory sites, and enhance the extravasation of serum. Another report showed that oxidants can suppress the function of β-adrenergic receptors (52), which may enhance smooth muscle contraction. There is increasing evidence that ROS can activate the DNA binding capacity of nucleus factor kappa B (NF-kB) which mediates various proinflammatory cytokines, adhesion molecules, and chemokines (5, 7, 93). NF-kB can control the expression of various proinflammatory mediators. These mediators are thought to be important in the inflammation of airways in asthmatic patients. Therefore, the suppression or depletion of oxidants by specific inhibitors or scavengers may be a potent therapeutic target for the suppression of airway inflammation in asthmatic patients in the future.

Antioxidant system in lungs and bronchial asthma

Since the lungs are exposed to various types of oxidative stress, both endogenous and exogenous, the antioxidant system is well developed in lungs. Table 1 shows the antioxidant system in human lungs. There is increasing evidence that there are alterations of the antioxidant capacity in asthmatic individuals

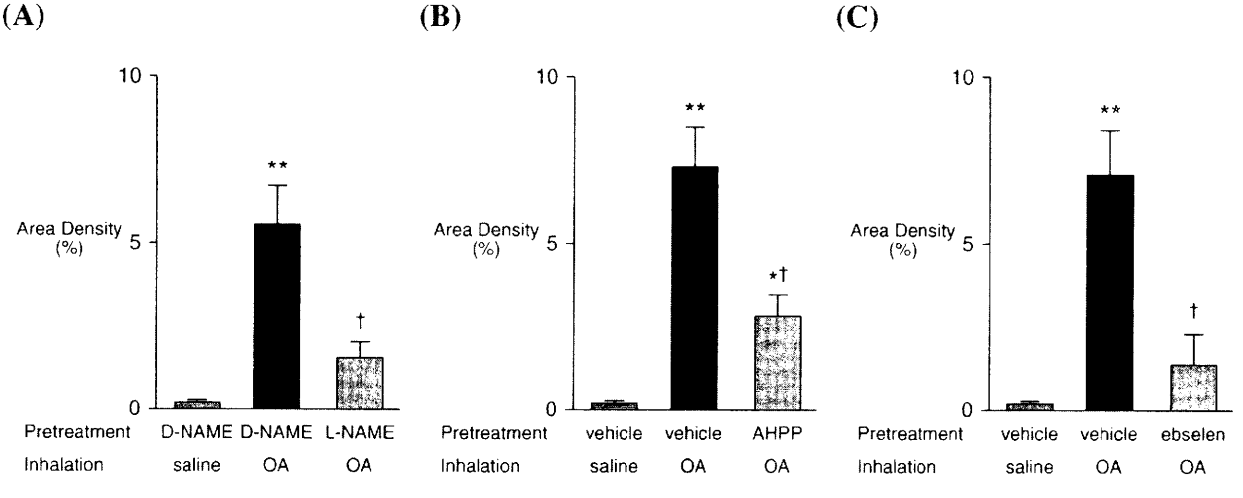

FIG. 1. Effects of nitric oxide synthase (NOS) inhibitor, xanthine oxidase (XO) inhibitor, and peroxynitrite scavenger on airway microvascular permeability during late allergic response (LAR). *Vertical axes* showed percentages of leaky microvasculature in trachea of guinea pigs. The NOS inhibitor, N^6-nitro-L-arginine methyl ester (L-NAME), significantly suppressed the microvascular permeability during LAR [(**A**) *open bar*: vehicle-treated saline-exposed animals; *closed bar*: inactive enantiomar, N^6-nitro-D-arginine methyl ester (D-NAME)-treated antigen-challenged animals; *hatched bar*: L-NAME-treated antigen challenged animals]. The XO inhibitor, {4-amino-6-hydroxypyrazolo(3,4-d)pyrimidine}(AHPP), significantly suppressed the microvascular permeability during LAR [(**B**), *open bar*: vehicle-treated saline-exposed animals; *closed bar*: vehicle-treated antigen-challenged animals; *hatched bar*: AHPP-treated antigen challenged animals]. The peroxynitrite scavenger, ebselen, significantly suppressed the microvascular permeability during LAR [(**C**) *open bar*: vehicle-treated saline-exposed animals; *closed bar*: vehicle-treated antigen-challenged animals; *hatched bar*: ebselen-treated antigen challenged animals). $*p < 0.05$, $**p < 0.01$ compared with vehicle-treated saline-exposed group. $+p < 0.05$ compared with each inhibitor or scavenger-treated antigen-challenged group. Excerpted with permission Sugiura H, Ichinose M, Oyake T *et al. Am J Respir Crit Care Med* 1999; 160: 663–671. Copyright 1999 American Thoracic Society.

TABLE 1. ANTIOXIDANTS IN HUMAN LUNGS

Antioxidants	*Localization*
Cu, Zn-superoxide dismutase	Cytosol
Mn-superoxide dismutase	Mitochondria
Extracellular-superoxide dismutase	Epithelial lining fluid
Catalase	Cytosol, Alveolar space
Glutathione	Cytosol, Epithelial lining fluid
Glutathione peroxidase	Cytosol
Heme oxygenase-1, -2	Cytosol
Ascorbic acid	Extracellular
α-Tocophenol	Cell membrane
β-Carotene	Cell membrane

as compared with healthy subjects. Additionally, there are discrepancies in the expression of antioxidants in bronchial asthma. For instance, the expression of some antioxidants such as GSH and heme oxygenase was reported to be increased in bronchial asthmatic patients compared with healthy subjects, whereas other antioxidants such as SOD and glutathione peroxidase were decreased (84). The reasons for such discrepancies have not been elucidated. The relationship between antioxidant and bronchial asthma is reviewed in the following section.

Glutathione (GSH). GSH has an SH residue and reacts with oxygen radicals. GSH is also a low molecular weight, water-soluble radical scavenger, and a high concentration of GSH (~500 μM) exists in the epithelial lining fluid of the airways (14). The ratio of GSSG, the oxidized form of GSH, to 2GSH (GSSG/2GSH) can serve as a good indicator of the cellular redox state (77, 84). This ratio in GSH may be determined by the rates of hydrogen peroxide reduction by glutathione peroxidase and GSSG reduction by glutathione peroxidase (84). Thus, antioxidant enzymes play a critical role in the maintenance of the cellular reductive potential. In the airways of asthmatic patients, the levels of GSH are increased in BALf, suggesting that GSH production may be upregulated to protect the lungs from excessive oxidative stress in asthmatic patients (99). Because excessive oxidative stress enhances the airway inflammation, GSH could be a therapeutic target for bronchial asthma. A previous report showed that inhalation of GSH using an ultranebulizer induced bronchoconstriction in mild asthmatic patients; the low pH (3.0) of the GSH solution caused bronchoconstriction in mild asthmatics (62). The effect of GSH on asthmatic patients is not well known using other routes of GSH administration.

Superoxide dismutase (SOD). SOD is present in essentially every cell in the body and has been shown to play an important role in protecting cells and tissues against superoxide anion (66, 67). This antioxidant enzyme decomposes superoxide anion into hydrogen peroxide and oxygen as shown in the following equation:

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Three types of SODs have been reported (84). All the forms of SODs act by a common mechanism of dismutation of superoxide anion to the less potent hydrogen peroxide. One of the three forms of SODs is Cu,ZnSOD (66, 84). Its molecular weight is 17–28 kDa and it is mainly located in the cytosol. In lungs, it is localized in the bronchial and alveolar epithelium, macrophages, fibroblasts, and pneumocytes. Another form of SOD is MnSOD (84). The molecular weight of this form of SOD is 88 kDa and it is mainly located in mitochondria. It is localized in bronchial epithelium, macrophages, neutrophils, endothelial cells, vascular smooth muscle cells, and pneumocytes. The other form of SOD is an extra-cellular SOD (EC-SOD) (38). Its molecular weight is 135 kDa and it is abundantly present in blood vessels and the airways. It is a secretory, tetrameric glycoprotein and requires Cu and Zn for its activity. The expression of EC-SOD is induced by interferon-γ and depressed by tumor necrosis factor (TNF-α), transforming growth factor-β (TGF-β), and interleukin-1α (IL-1α) in fibroblasts (61). EC-SOD has also been found to be expressed by bronchial epithelial cells, type II pneumocytes, endothelial cells, and alveolar mecrophages (102). Our previous study showed that superoxide anion can cause airway hyper-responsiveness in an animal model (46). Intratracheal administration of xanthine-xanthine oxidase induces airway hyper-responsiveness in cat (46). This airway hyper-responsiveness was suppressed by pretreatment with Cu,ZnSOD, suggesting that the generation of superoxide anion is associated with the formation of airway hyper-responsiveness, which is a major feature of bronchial asthma (8). We also previously showed that exposure to ozone-induced airway hyper-responsiveness to methacholine and SOD reversed the increased airway hyper-responsiveness in cat (106), suggesting that superoxide anion is related to the ozone-induced airway hyper-responsiveness. A previous study showed that the activity of SOD was decreased in the epithelial cells from asthmatic patients compared with healthy subjects (100). Furthermore, the activity of SOD in the BALf from asthmatic patients was decreased compared with healthy subjects (100). The decreased SOD activity may prolong the survival of superoxide anion in the lung tissues. Because superoxide can induce injury of the airway epithelial cells and airway microvascular hyperpermeability, the decreased SOD activity may be associated with the airway inflammation and formation of hyper-responsiveness in asthmatic patients.