Potential role of pentosidine on susceptibility to small airway closure in elderly and smoking asthma

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Summary

Background: Small airway closure in asthma is determined by a complex interaction of structural and functional characteristics including lung elastic recoil. Recently, we determined that loss of elastic recoil might be attributable to pentosidine level in the airways. This study was designed to investigate the influences of aging and smoking on small airway closure in asthma.

Methods: Sixty-one patients with asthma (20 non-smoking young adult, 23 non-smoking elderly, and 18 smoking young adult) and 36 control subjects (12 non-smoking young adult, 11 non-smoking elderly, and 13 smoking young adult) were included. We assessed airway responses during methacholine provocation and calculated the closing index. In addition, we measured pentosidine levels in induced sputum from all study subjects.

Results: Pentosidine levels in induced sputum were markedly higher in asthmatic patients than in controls. In control subjects, the intergroup differences in pentosidine level among 3 subgroups were significant. Similarly, pentosidine levels were significantly higher in non-smoking elderly and smoking young adult asthmatics than in non-smoking young adult asthmatics. There was no significant difference in pentosidine levels between non-smoking elderly and smoking young adult asthmatics. The closing index was also significantly higher in non-smoking elderly and smoking young adult asthmatics than in non-smoking young adult asthmatics. Moreover, pentosidine levels in non-smoking elderly and smoking young adult asthmatics were closely correlated with closing index.

Abbreviations: delta N2, slope of the nitrogen alveolar plateau; DRS, dose–response slope; DTT, dithiothreitol; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; IQR, interquartile range; NO, nitric oxide; PC20 methacholine, provocative concentration causing a 20% fall in FEV1 with methacholine.

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Introduction

Asthma is a chronic inflammatory airway disease characterized by variable airway obstruction attributed to an underlying inflammatory process and airway remodeling [1]. In general, the pathophysiology of asthma has been based on these inflammatory and structural changes occurring predominantly in the large airways [2]. However, these histopathologic changes also occur in the small airways, resulting in functional consequences such as small airway closure [3]. Increased small airway closure has been associated with a greater risk of severe asthma exacerbations and correlated with the severity of asthma [4]. Small airway closure in asthma is determined by a complex interaction of structural and functional characteristics including the thickness and elastic properties of the airway wall [5]. Several studies have determined that reduced elastic recoil is primarily responsible for small airway closure in asthma [6–8]. These findings indicate that the relative contribution of reduced elastic recoil to small airway closure is of high importance [9]. Interestingly, reduced elastic recoil has been observed in stable, asymptomatic asthmatic patients as well as in patients with severe exacerbations of the disease. Moreover, it is also reported that even asthmatic patients with no actual airway obstruction have reduced elastic recoil [10].

Pentosidine is well established as an intermolecular cross-linking type of advanced glycation end products, and it accumulates with aging in various connective tissues including collagen [11–14]. It is formed through a series of non-enzymatic reactions between glucose and proteins resulting in a highly stable cross-linked product. One of the contributing factors to its production is oxidative stress. Although all proteins are prone to pentosidin formation, deleterious pentosidine accumulation occurs in tissues with low turnover, including collagen. This accumulation subtly alters collagen structure and function, increasing stiffness in arteries, skin, bone, and lung [15,16]. In human studies, pentosidine has also been found to be responsible for collagen cross-linking in lung tissues [17]. Recently, we demonstrated that pentosidine levels in induced sputum were significantly higher in asthmatic patients than in normal controls, and that loss of elastic recoil might be attributable to pentosidine level in the airways [18].

An aging population will result in increased prevalence of elderly asthma; therefore, it is important to clarify the pathophysiological features of this condition. To date, difficulties have been encountered in differentiating asthma and COPD [19]. Cigarette smoking is a major diagnostic confounder in elderly populations, and published descriptions of elderly asthma are derived from cohorts that include smokers [20]. Because of the confounding effects of smoking, these descriptions might not truly reflect elderly asthma. A previous study reported that small airway closure in elderly asthmatics was increased compared with younger asthmatics [7]. Although age-related loss of elastic recoil is likely the main cause of this phenomenon, the precise mechanism remains undetermined. In order for a population of elderly asthmatic patients to serve as a model in which to examine small airway closure, the confounding effects of smoking must be eliminated. Therefore, this study was designed to investigate the influences of aging and smoking on susceptibility to small airway closure in asthma.

Methods

Study subjects

Sixty-one patients with asthma (20 non-smoking young adult, 23 non-smoking elderly, and 18 smoking young adult) and thirty-six control subjects (12 non-smoking young adult, 11 non-smoking elderly, and 13 smoking young adult) were included in this study. Young adult subjects were aged 20–40 years, and elderly subjects were more than 60 years of age. Smokers were aged 20–40 years, and had more than a 10 pack-year history (control smokers, range: 10–19 pack-years; asthmatic smokers: 10–21 pack-years). All control subjects were volunteers who had no history of lung diseases. All asthmatic patients had been newly diagnosed with asthma at the respiratory outpatient clinics of our university hospital on entry into this study. All asthmatics had: (1) episodic cough, wheeze and dyspnea; (2) normal chest roentgenograph; (3) reduction of forced expiratory volume in 1 s (FEV1) in case of asthma attack and increase of 20% or greater in FEV1 in response to a bronchodilator; (4) airway hyper-responsiveness to methacholine. In addition, all of them were atopy; total serum immunoglobulin E (IgE) levels were more than 250 IU/mL, and one or more common aeroallergens were determined by specific IgE. At the time of the study, all asthmatic patients were solely receiving salbutamol for as-needed relief of symptoms without controller medications (i.e., long-acting β2-agonists, leukotriene modifiers, and oral or inhaled corticosteroids). Exhaled nitric oxide (NO) levels were measured with a chemiluminescence analyzer with a resolution of 1 parts per billion (ppb) in accordance with the American Thoracic Society standards [21]. All asthmatic patients were clinically stable, and none had a history of respiratory infection for at least the 4-week period preceding the study. All subjects provided written informed consent for participation in the study, which was approved by the Ethics Committee of Osaka City University.

Conclusions: We determined the correlation of pentosidine level with susceptibility to small airway closure in elderly and smoking asthmatics. Our results might facilitate the understanding of elderly and smoking asthma.

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Pulmonary function test

All study subjects underwent pulmonary function tests just before sputum induction, by using a CHESTAC-8800 unit (Chest, Tokyo, Japan) according to the recommendations of the American Thoracic Society/European Respiratory Society [22, 23]. Forced vital capacity (FVC) and FEV₁ were determined. The best of 3 consecutive attempts in spirometric measurements was recorded.

Methacholine provocation test

The methacholine provocation test was performed in all study subjects. The test was performed as we previously described [24]. The provocative concentration causing a 20% fall in FEV₁ with methacholine (PC20 methacholine) was calculated by interpolating between 2 adjacent data points when the FEV₁ decreased by >20%. Spirometric measurements of FEV₁ and FVC were made after each concentration step of methacholine challenge, with FVC maneuvers continued for a minimum of 6 s and until a clean concentration step of methacholine challenge, with FVC to airway narrowing (DRS[FEV₁]) and sensitivity to airway challenge divided by the dose in micromoles. Thus, sensitivity measurements of FEV₁ and FVC were made after each points when the FEV₁ decreased by was calculated by interpolating between 2 adjacent data

Sputum induction and processing

Sputum induction was performed as we previously described [25]. The slides were prepared using a cytoospin (Cytoospin 3: Shandon, Tokyo, Japan) and stained with May-Grunwald–Giemsa stain for eosinophil counts. Pentosidine levels in sputum samples were measured using commercial enzyme-linked immunosorbent assay kits (Fushimi Pharmaceutical Co., Marugame, Japan). Preliminarily, we examined the reproducibility and found no detrimental effects of dithiothreitol (final concentration of 1 mM) (WAKO Pure Chemical Industries Ltd, Osaka, Japan) on measurement of pentosidine levels in induced sputum. In this assay system, the limit of detection for pentosidine was 0.5 ng/mL.

Statistical analysis

All data are presented as median and interquartile range (IQR). When multiple comparisons of non-parametric data were performed between groups, significant intergroup variability was first established using the Kruskal–Wallis test. The Mann–Whitney U test was then used for intergroup comparisons. The significance of correlation was evaluated by determining Spearman’s rank correlation coefficients. A p-value <0.05 was considered significant.

Results

Clinical characteristics of study subjects

The clinical characteristics of 61 patients with asthma and 36 control subjects are shown in Table 1. Compared with control subjects, asthmatic patients showed greater airway obstruction. Furthermore, the percentage of eosinophils in induced sputum and exhaled NO level were also significantly higher in asthmatic patients than in controls. No significant differences in baseline lung function and sputum eosinophils could be observed among 3 subgroups of asthmatic patients.

Pentosidine levels in control subjects and asthmatic patients

Pentosidine levels in induced sputum were markedly higher in asthmatic patients than in controls (asthmatics: 23.9 [18.6–29.8] ng/mL; controls: 3.3 [1.6–9.4] ng/mL; p < 0.001). In control subjects, the intergroup differences in pentosidine level among 3 subgroups were significant (non-smoking young adult: 1.9 [0.8–3.0] ng/mL; non-smoking elderly: 9.6 [6.7–10.2] ng/mL; smoking young adult: 5.3 [3.8–6.2] ng/mL) (Fig. 1). Pentosidine levels in non-smoking elderly and smoking young adult controls were significantly higher than those in non-smoking young adult controls. Similarly, the intergroup differences in pentosidine level among 3 subgroups of asthmatics were also significant (non-smoking young adult: 16.5 [15.2–18.3] ng/mL; non-smoking elderly: 27.3 [22.8–34.4] ng/mL; smoking young adult: 26.8 [25.4–30.3] ng/mL). There was no significant difference between non-smoking elderly and smoking young adult asthmatics. In contrast, pentosidine levels in non-smoking elderly and smoking young adult asthmatics were significantly higher than in non-smoking young adult asthmatics.

Airway closure in control subjects and asthmatic patients

All asthmatic patients had airway hyperresponsiveness to methacholine (PC20 methacholine <8 mg/mL) (Table 2). PC20 methacholine showed similar levels among 3 subgroups of asthmatics. Maximum changes in FEV₁ and FVC after methacholine provocation were also similar among 3 subgroups of asthmatics. Both sensitivity to airway narrowing (DRS[FEV₁]) and sensitivity to airway closure (DRS[FVC]) were significantly higher in asthmatic patients than in controls. DRS[FEV₁] did not differ significantly among 3 subgroups of controls and asthmatic patients. Although DRS[FVC] was similar in 3 subgroups of controls, the non-smoking elderly and smoking young adult asthmatics showed significantly higher levels than non-smoking young adult asthmatics. However, there was no significant difference in DRS[FVC] between non-smoking elderly and
FEV1 and FVC implies that the contribution of airway closure to the change in FVC throughout the methacholine challenge in all study subgroups of control subjects. In addition, the actual levels of airway inflammation (% eosinophils in induced sputum and exhaled NO levels) were also not significantly correlated with closing index.

Table 1 Clinical characteristics of study subjects.  

<table>
<thead>
<tr>
<th>Control subjects</th>
<th>Asthmatic patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject No. (M/F)</td>
<td>12 (5/7) 11 (3/8)</td>
</tr>
<tr>
<td>FVC/FVC (%)</td>
<td>0.88 [81–90] 0.81 [78–88]</td>
</tr>
<tr>
<td>Sputum eosinophils (%)</td>
<td>0.1 [0–0.3] 0.2 [0–0.3]</td>
</tr>
</tbody>
</table>

Definition of abbreviations: FEV1 = forced expiratory volume in one second; FVC = forced vital capacity; NO = nitric oxide.

All values are median [ICR].

a p < 0.01 compared with non-smoking young adult controls.

b p < 0.01 compared with non-smoking elderly controls.

c p < 0.05 compared with non-smoking young adult asthmatics.

However, pentosidine level was not significantly correlated with closing index in non-smoking young asthmatics and 3 subgroups of control subjects. In addition, the actual levels of airway inflammation (% eosinophils in induced sputum and exhaled NO levels) were also not significantly correlated with closing index.

Discussion

Increased small airway closure in asthma may lead to a mechanical disadvantage and contribute to dyspnea and limitations of physical activity. From this viewpoint, small airway closure is a clinically important profile of the disease severity. However, small airway closure is difficult to measure directly and, therefore, has been estimated indirectly using a range of physiological techniques. We also assessed spirometric parameters during methacholine provocation as simple surrogate measures of small airway closure. Using this method, we could evaluate the extent of airway narrowing and airway closure separately, and represented the extent of small airway closure as the closing index. To date, it is not clear whether the extent of small airway closure differs between asthmatic and non-asthmatic subjects. Using radionuclide imaging, King et al. found no difference in the extent of small airway closure between asthmatic and non-asthmatic subjects [26]. In this study, we also found that closing index was similar in asthmatic and non-asthmatic subjects of aged 20–40 years.

Smokers with asthma have poorly controlled symptoms, impaired therapeutic responses to corticosteroids, and increased rates of health care utilization compared with non-smokers with asthma [27]. In this study, we found that smoking itself is an essential causal factor for increased small airway closure in asthmatic subjects. The effects of smoking might contribute to the accelerated loss of lung elastic recoil, resulting in an increased tendency to small airway closure. Elderly asthma is often assumed to include 2 subgroups: those who have had asthma from childhood (long-standing asthma) and those who develop asthma in old age (late-onset asthma). Because we included only the latter in this study, it was
not necessary to consider the effects of asthma duration on small airway closure. In normal aging, there are age-related structural changes in the respiratory system. With increased age, elastic fibers in the lung parenchyma decrease, and alter the elastic properties of the airways. Thus, aging itself has been associated with loss of elastic recoil, resulting in mechanical disruption [28]. However, the former hypothesis is that elastic recoil is lost with ageing because of changes in the spatial arrangement and/or crosslinking of the elastic fiber network or because of the presence of a pseudoelastin [29]. The major lung extracellular matrix components are collagens, elastic fibers, proteoglycans, fibronectin and tenascin. Collagen and elastin are the main proteins in the extracellular matrix that make up the framework of the lung parenchyma and most important in determining the mechanical properties [30]. In particular, collagen is the most abundant component. Therefore, it is plausible that the abnormal cross-linking of collagen mediated by pentosidine might contribute to lung elastic recoil. Elderly asthma is supposed to prominently involve small airway closure [31]. Milanese et al. previously found that small airway closure exists in older asthmatics compared with younger asthmatics [32]. In this study, weals of o ut th a t aging is a causal factor for increased small airway closure in asthmatic subjects. These findings suggest that elderly asthma may have an increased small airway closure via age-related loss of elastic recoil. Thus, smoking and elderly asthmatics had markedly accelerated the susceptibility to small airway closure compared with younger and non-smoking asthmatics.

The precise mechanism of increased small airway closure in smokers and elderly subjects with asthma is uncertain. In particular, there is no clear consensus as to what constitutes small airway closure in asthmatic patients. It is plausible that multiple mechanisms may lead to small airway closure in elderly and smoking asthmatics. We demonstrated for the first time that pentosidine levels were significantly higher in smoking young adult controls compared with non-smoking young adult controls, and that those levels were further increased in smoking young adult asthmatics. Similarly, pentosidine levels were significantly higher in non-smoking elderly asthmatics.
than in non-smoking elderly non-asthmatics. A noteworthy finding is that pentosidine levels in smoking young adult and non-smoking elderly asthmatics were closely correlated with closing index. Pentosidine levels in non-smoking elderly and smoking young adult controls were significantly higher than those in non-smoking young adult controls. However, its levels in elderly and smoking controls were considerably low compared to 3 subgroups of asthmatic patients. It is supposed that pentosidine levels above a certain threshold might be required to affect closing index. Moreover, we did not find the significant correlation between pentosidine level and % eosinophils in induced sputum or exhaled NO level in 3 subgroups of asthmatic patients. Thus, long-term pentosidine accumulation in the airways was not significantly correlated with the severity of current airway inflammation. On the basis of these findings, the present study clearly showed that the susceptibility to small airway closure is associated with airway pentosidine level. We previously found an increase in the slope of the nitrogen alveolar plateau (delta N2) with aging, and that values of delta N2 in each age range were significantly higher in asthmatics than in normal controls [33]. Moreover, pentosidine level was closely correlated with delta N2 value in asthmatic patients. These findings suggest that high levels of pentosidine may be associated with small airway closure or near closure. The aging lung may undergo prominent changes in structure and function, including loss of elastic recoil. Because elastic recoil may be a limiting factor in increased small airway closure during bronchoconstriction, age- and smoking-related loss in elastic recoil may result in increased small airway closure.

The present study has several limitations. First, this study revealed an association between pentosidine level and extent of small airway closure, but did not investigate the causality of this association. Longitudinal studies will be required to determine direct causal relationships between these factors. Moreover, it is also important to know the relationship between airway remodeling and pentosidine accumulation in the airways. Second, we estimated the extent of small airway closure by using only spirometry. The results should be assessed using other techniques such as nitrogen washout and radionuclide imaging.

In summary, we revealed a significant association between airway pentosidine level and small airway closure in elderly and smoking asthmatics, and suggest that increased airway pentosidine levels evoked by smoking and aging may lead to small airway closure associated with loss of lung elastic recoil. These findings suggest that aging and smoking may independently accelerate the susceptibility to small airway closure in asthmatic subjects. Our results will encourage more studies to assess the relationship between airway pentosidine level and mechanical airway properties in smoking and elderly asthmatics. This study will provide new insights into small airway closure in asthma, and may be of great use in understanding the pathogenesis of elderly and smoking asthma.

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**Conflict of interest statement**

All authors (HK, SK, YT, KA, and KH) of this manuscript have no actual or potential conflicts of interest to disclose.

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Author contributions: Dr. Kanazawa had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Dr. Kanazawa: contributed to study design; data collection and analysis; manuscript writing and preparation; and reviewing, editing, and approving the manuscript. Dr. Kyoh: contributed to data analysis, reviewing, editing, and approving the manuscript. Dr. Tochino: contributed to data analysis and reviewing, editing, and approving the manuscript. Dr. Asai: contributed to data collection and reviewing, editing, and approving the manuscript. Dr. Hirata: contributed to study design; participant recruitment; data analysis; manuscript writing and preparation; and reviewing, editing, and approving the manuscript.

**References**

Role of pentosidine on small airway closure


