
◎原 著

Comparison of pathophysiological changes of asthma between younger and older patients with asthma in relation to long-term cigarette smoking

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Abstract : Influence of long-term cigarette smoking on the pathophysiological changes of the disease was compared between younger and older patients with asthma. 1. The frequency of positive RAST score against inhalant allergens was significantly higher in ex-smokers than in never-smokers in older patients with asthma, but not in younger subjects. 2. Ventilatory parameters such as %FVC and FEV_{1.0%} were not significantly different between ex-smokers and never-smokers either in younger or older patients, although a significant difference in these values was observed between younger and older subjects. 3. The %DLco was significantly lower in ex-smokers than in never-smokers both in younger and older patients. 4. The parameters associated with hyperinflation such as %RV and %LAA of the lungs on HRCT were significantly higher in older patients than in younger subjects, and also significantly larger in ex-smokers than in never-smokers in older patients, but %RV was not different between ex-smokers and never-smokers in young subjects. 5. Bronchial hyperresponsiveness (BH) was significantly more increased in ex-smokers than in never-smokers in older patients, but not in younger subjects. There was a significant difference in BH between younger and older patients. 6. LTB₄ generation was significantly larger in ex-smokers than in never-smokers in older patients, and LTC₄ generation was more increased in ex-smokers both in younger and older subjects. The results show that significant differences between ex-smokers and never-smokers were observed in %DLco, LTC₄ generation and %LAA both in younger and older subjects, and in IgE antibodies production, %RV, BH and LTB₄ generation only in older subjects. In contrast, the values of %FVC and FEV_{1.0%} were not different between ex-smokers and never-smokers either in younger or older subjects. A significant difference between younger and older subjects was found in all other parameters except %DLco and LTC₄ generation.

Key words : cigarette smoking, %RV, %LAA of the lungs on HRCT, asthma, bronchial hyperresponsiveness

Introduction

Airway inflammation is a characteristic feature of asthma, in which inflammatory cells such as macrophages, lymphocytes, eosinophils, neutrophils, and basophils migrate into allergic reaction sites induced by immunoglobulin (Ig) E-mediated allergy¹⁻³), and bronchial hyperresponsiveness related to an increase in the generation of leukotriene B4 (LTB4) by leucocytes is observed⁴). Regarding pulmonary function in asthma, obstructive ventilatory dysfunction, slight decrease in the diffusing capacity for carbon monoxide (DLco), and hyperinflation of the lungs expressed by residual volume (RV) and %low attenuation area (LAA) of the lungs on high-resolution computed tomography (HRCT) are often observed in patients with asthma⁵⁻⁷), particularly in the elderly.

It has been shown that cigarette smoking is closely related to the onset mechanisms of chronic obstructive pulmonary disease (COPD)⁸). An association between smoke exposure and the induction⁹) or exacerbation of asthma has also been reported¹⁰). Current smoking has been shown to increase asthma severity¹¹), and higher incidences of asthma are found in current and former smokers, compared with never-smokers¹²⁻¹⁴). Our previous studies have shown that %LAA < -950 Hounsfield Units (HU) of the lungs on HRCT and %RV were significantly larger in asthmatics with a smoking history than in those without¹⁵). The results suggest that cigarette smoking enhances the production of IgE antibodies, bronchial hyperresponsiveness, and generation of LTB4 by leucocytes in elderly asthmatics. Furthermore, it has been shown that increased hyperinflation or emphysematous changes of the lungs expressed by increased %LAA of the lungs on HRCT, closely related

to %RV, was frequently observed in ex-smokers compared with never-smokers.

The present study was performed to examine whether some differences are present in the influences of cigarette smoking on the pathophysiology of asthma between younger and older patients with asthma.

Subjects and Methods

The subjects in this study were 78 patients (33 females and 45 males) with asthma. The subjects were divided into two groups according to their age (23 younger patients under age 49 and 55 older subjects over age 65), and further divided into two groups according to smoking history (30 with smoking history and 48 without). The mean age of younger patients with asthma was 46.4 years in 5 ex-smokers, and 36.4 years in 18 never-smokers. The mean age of older patients was 72.0 years in ex-smokers, and 71.5 years in never-smokers.

All subjects with asthma had episodic symptoms of wheezing and coughing and experienced symptomatic relief and reversible airway response with an accompanying increase exceeding 15% in forced expiratory volume in 1 s (FEV₁) upon treatment with β_2 -adrenergic agonist.

Spirometry was performed by means of a CHESTAC 33 (Chest Co.) linked to a computer. The following measurements were performed on all subjects: forced vital capacity (FVC), FEV_{1.0%} (FEV₁/FVC). The RV was measured using body plethysmography (Autobox 2800, Chest Co.). The DLco was measured by the single-breath technique using CHESTAC 33. FVC, FEV₁, RV, and DLco measurements for each patient were expressed as percentage of predicted values.

Bronchial hyperresponsiveness to methacholine was measured by an Astograph (TSK 6100, Chest Co.). Different concentrations of

methacholine (49, 98, 195, 390, 781, 1,563, 3,125, 6,250, and 12,500 $\mu\text{g}/\text{ml}$) were prepared for bronchial challenge according to the method used by Chai et al.¹⁶. The increase of total respiratory resistance (Rrs) after methocholine inhalation was measured by the oscillation method. A methacholine concentration causing a significant increase in Rrs was assessed as Cmin (minimum concentration). All medications were stopped 12 hours prior to examination.

The %LAA < -950 HU of the lungs on HRCT was measured using a Toshiba Xpeed scanner (Toshiba, Co.) with 2 mm collimation, scanning time 2.7s, voltage 120 kVp, and current 200 mA. Maximal inspiratory HRCT scan was obtained at the following three anatomic levels, as described by Miniati et al.¹⁷: (1) top of the aortic arch, (2) origin of the lower lobe bronchus, and (3) 3 cm above the top of the diaphragm. The results were expressed as the mean of %LAA at the three anatomical levels.

The generation of leukotrienes, LTB₄ and leukotriene C₄ (LTC₄), by peripheral leucocytes was assessed as described previously^{3, 4, 18}. Five milliliters of 6% dextran (molecular weight ~ 200,000 kDa) (Nacalai Tesque Inc.) were added to 20 mL heparinized peripheral blood, and the resultant mixture was incubated for 1 hour at room temperature. The leucocyte-rich plasma supernatant was then removed and used. The number of cells was adjusted to 5×10^6 cells/mL in Tris CM buffer, and the cells were then incubated with 1 μg calcium ionophore A23187 (Sigma, St Louis, Mo, USA) for 15 min at 37°C. After a 4x volume of prechilled ethanol (final, 80% ethanol) was added, the mixture was centrifuged at 1870 x g for 30 min. A syringe filter (Toyo Roshi Co.) was used to draw the supernatant, and the filtrate was decompressed and dried to a solid. Quantification of LTB₄ and LTC₄ was performed by high-performance

liquid chromatography (HPLC) and ultraviolet (UV) spectroscopy, following the method of Lam et al.¹⁹. Quantities of LTB₄ and LTC₄ were expressed as nanograms per 5×10^6 cells.

Serum IgE levels were evaluated by radioimmunosorbent test (RIST), and IgE antibodies specific to aeroallergens such as house dust mites, pollens, moulds and animal danders were measured using the Pharmacia CAP system (Pharmacia Diagnostic AB, Uppsala, Sweden).

Statistically significant differences of the mean were estimated using the unpaired Student's t test. A p value of < 0.05 was regarded as significant.

Results

The frequency of patients with serum IgE level more than 200 IU/ml was higher in younger patients than in older subjects, but this was not significant. Any significant differences in serum IgE levels were not found between ex-smokers and never-smokers either of younger or older subjects. The frequency of patients with a positive RAST score against inhalant allergens was significantly higher in younger patients than in older subjects in the never-smokers ($p < 0.05$), and also significantly higher in ex-smokers than in never-smokers in the older subjects ($p < 0.05$) (Table 1).

Table 1. Serum IgE and positive rate of RAST score against inhalant allergens in patients with asthma in relation to cigarette smoking

	Age	N	Serum IgE* (IU/ml)	positive RAST ** (%)
Younger	Ex	5	4/5 (80.0%)	4/5 (80.0%)
	Ne	18	10/18 (55.6%)	14/18 ^a (77.8%)
Older	Ex	25	11/25 (44.0%)	21/25 ^b (84.0%)
	Ne	30	10/30 (33.3%)	12/30 ^{ab} (40.0%)

*Frequency of patients with serum IgE more than 200 IU/ml. **positive rate of RAST score against inhalant allergens. Ex:ex-smokers, Ne:never-smokers. a and b:p<0.05.

The values of %FVC and FEV_{1.0%} were significantly higher in younger patients than in older subjects both of ex-smokers and never-smokers. However, a significant difference in these values was not observed between ex-smokers and never-smokers either in younger or older subjects (Fig. 1, 2). The %DLco value showed a

tendency to decrease with aging. The value was significantly larger in never-smokers than in ex-smokers both in younger ($p < 0.05$) and older patients ($p < 0.001$) (Fig. 3).

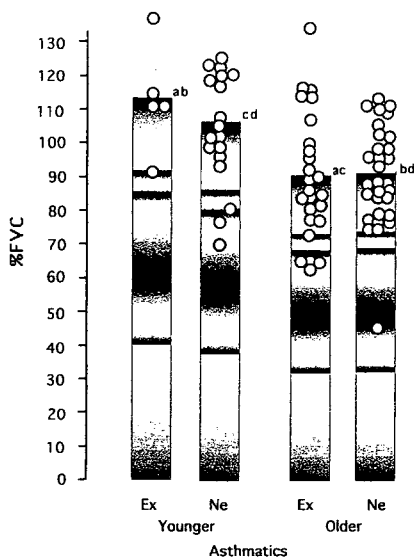


Fig.1. Comparison in %FVC between younger and older patients with asthma in relation to cigarette smoking. Ex: ex-smokers, Ne: never-smokers. a and b: $p < 0.05$, c: $p < 0.01$, d: $p < 0.02$.

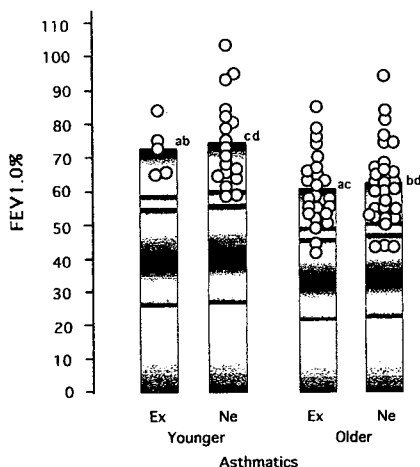


Fig.2. Comparison in FEV_{1.0%} between younger and older patients with asthma in relation to cigarette smoking. Ex: ex-smokers, Ne: Never-smokers. a: $p < 0.05$, b, c and d: $p < 0.02$.

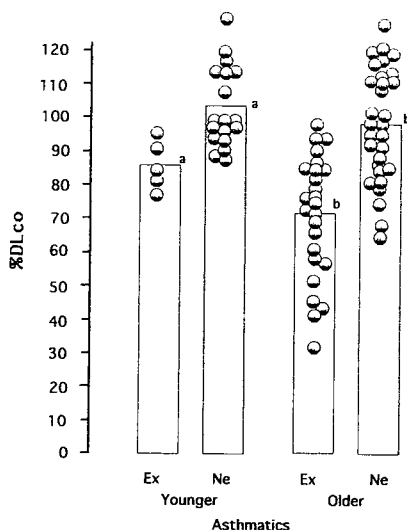


Fig.3. Comparison in %DLco between younger and older patients with asthma in relation to cigarette smoking. Ex: ex-smokers, Ne: never-smokers. a: $p < 0.05$, b: $p < 0.001$.

The %RV value was significantly more increased in ex-smokers than in never-smokers in the older patients ($p < 0.05$), but not in younger subjects. The value was significantly larger in older patients of ex-smokers than in never-smokers of younger subjects ($p < 0.02$) (Fig. 4). The %LAA of the lungs on HRCT was significantly more increased in older patients than in younger subjects both in ex-smokers and never-smokers, and significantly higher in ex-smokers than in never-smokers both in younger and older subjects (Fig. 5).

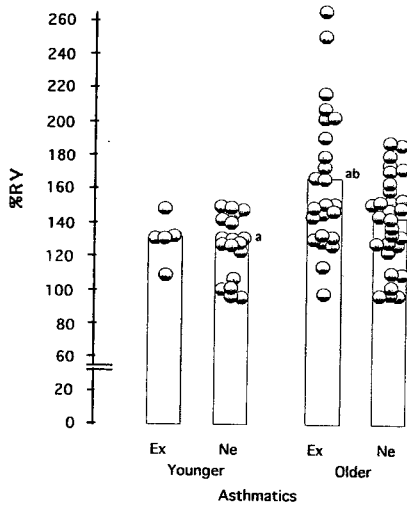


Fig. 4. Comparison in %RV between younger and older patients with asthma in relation to cigarette smoking. Ex: ex-smokers, Ne: never-smokers. a: $p < 0.02$, b: $p < 0.05$.

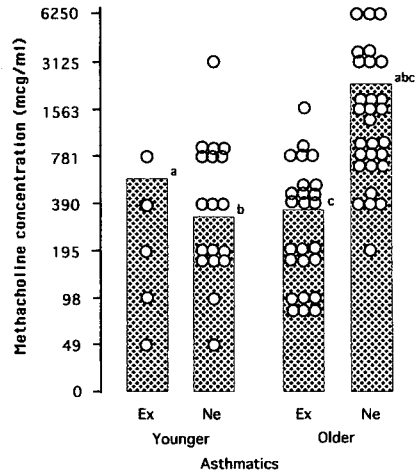


Fig. 6. Comparison in bronchial hyperresponsiveness to methacholine between younger and older patients with asthma in relation to cigarette smoking. Ex: ex-smokers, Ne: never-smokers. a: $p < 0.01$, b: $p < 0.02$, c: $p < 0.05$.

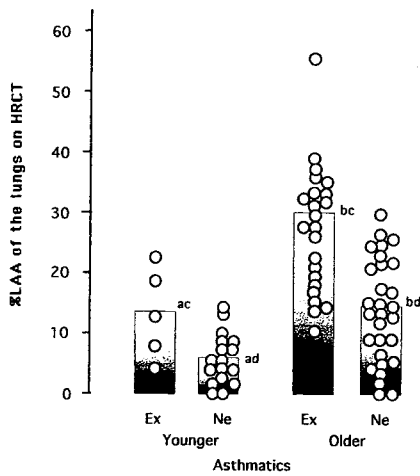


Fig. 5. Comparison in %low attenuation area (LAA) of the lungs on HRCT between younger and older patients with asthma in relation to cigarette smoking. Ex: ex-smokers, Ne: never-smokers. a and c: $p < 0.01$, b and d: $p < 0.001$.

Bronchial hyperresponsiveness to methacholine was significantly lower in never-smokers of older patients compared with the responsiveness in ex-smokers ($p < 0.01$) and never-smokers ($p < 0.02$) of younger subjects. A significant difference in the responsiveness was found between ex-smokers and never-smokers in older patients ($p < 0.05$), but not in younger subjects (Fig. 6).

The LTB₄ generation by leucocytes significantly lower in never-smokers of older patients than both in ex-smokers ($p < 0.001$) and never-smokers ($p < 0.001$) of younger subjects, and in ex-smokers of older subjects ($p < 0.001$). The LTB₄ generation was significantly more increased in ex-smokers than in never-smokers of older patients ($p < 0.001$), however, this difference was not found in younger subjects (Fig. 7). In contrast, LTC₄ generation by leucocytes was significantly more increased in ex-smokers than in never-smokers both in younger ($p < 0.05$) and older patients ($p < 0.001$). Any significant differences were not found in LTC₄ generation between younger and older subjects (Fig. 8).

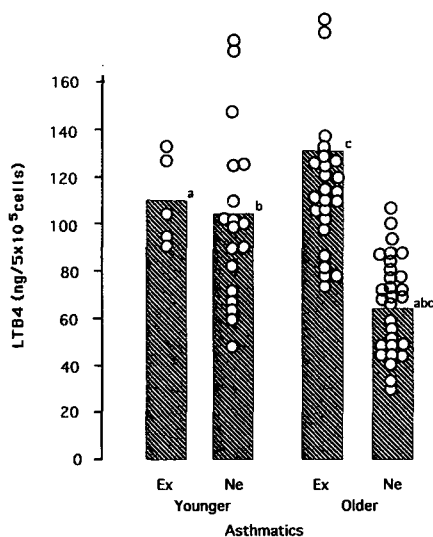


Fig. 7. Comparison in LTB₄ generation by leucocytes between younger and older patients with asthma in relation to cigarette smoking. Ex: ex-smokers, Ne: never-smokers. a, b, and c: $p < 0.001$.

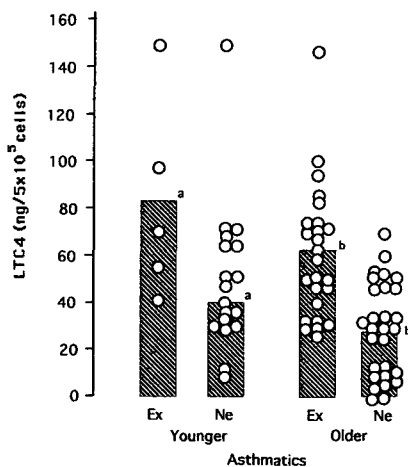


Fig. 8. Comparison in LTC₄ generation by leucocytes between younger and older patients with asthma in relation to cigarette smoking. Ex: ex-smokers, Ne: never-smokers. a: $p < 0.05$, b: $p < 0.001$.

Discussion

Several factors such as aging and cigarette smoking influences the pathophysiology of asthma. Cigarette smoke exposure is associated with the development of asthma in children^{20, 21}. The prevalence of physician-diagnosed asthma is

significantly larger in never-smokers with environmental tobacco smoke (ETS) exposure in childhood compared with that in non-exposed subjects²². Furthermore, higher incidence of asthma have been observed in current and former smokers, compared with never-smokers^{14, 23, 24}. Cigarette smoke-induced bronchial hyperresponsiveness is not associated with elevated IgE and eosinophilic inflammation¹¹. Cigarette smoke has an adjuvant effect on eosinophils, allergen-specific antibodies, and Th2 cytokines (IL-2 and IL-10) in adult mice previously sensitized injection with ovalbumin and aluminium hydroxide²⁵.

Our previous studies have shown that cigarette smoking enhances the production of IgE antibodies, bronchial hyperresponsiveness to methacholine, and generation of LTB₄ by leucocytes in elderly asthmatics^{15, 26}. In the present study, IgE antibodies (Ab) production, bronchial hyperresponsiveness, and generation of LTB₄ and LTC₄ by leucocytes were significantly more increased in ex-smokers compared with those in never-smokers of older patients, however, these increases except LTC₄ generation were not observed in ex-smokers of younger subjects. Furthermore, a difference in LTC₄ generation was not present between younger and older patients. The results suggest that IgE antibodies production and LTB₄ generation might be more strongly affected by aging than by cigarette smoking. Almost same tendency as in LTC₄ generation was observed in %DLco value: a significant difference between ex-smokers and non-smokers was present, but not between younger and older subjects (Table 2).

Table 2. Comparison in the pathophysiological features of asthma between younger and older patients

	Differences		
	between younger and older	ex- and never-smokers in younger	between never-smokers in older
production of IgE antibodies	+	-	+
%FVC	+	-	-
FEV _{1.0%}	+	-	-
%DL _{co}	-	+	+
%RV	+	+	+
%LAA on HRCT	+	+	+
BH*	+	-	+
LTB ₄	+	-	+
LTC ₄	-	+	+

BH: bronchial hyperresponsiveness

Hyperinflation of the lungs in asthma is expressed by %RV and %LAA of lungs on HRCT. The %RV value was significantly more increased in older patients than in younger subjects. The value also significantly more increased in ex-smokers than in never-smokers of older subjects, but not of younger subjects. The results show that %RV value was not so remarkably increased by cigarette smoking in younger subjects.

In contrast, %LAA value was significantly more increased in older patients than in younger subjects. The value was also significantly more increased in ex-smokers than in never-smokers both of younger and older subjects. A difference of %LAA from %RV was observed in younger patients with reference to cigarette smoking, as shown in Table 2. The difference in the results of %RV and %LAA in younger subjects might suggest that %LAA on HRCT expresses hyperinflation of lungs more accurately than %RV.

The values of %FVC and FEV_{1.0%} were affected by aging, but not by cigarette smoking both in younger and older subjects. In contrast, bronchial hyperresponsiveness was influenced not only by aging but also by cigarette smoking in older patients, but not in younger subjects. The results suggest that a decrease in bronchial hyperresponsiveness with aging is accelerated by cigarette smoking, which might be mediated

by an increased production of IgE antibodies.

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気管支喘息における長期間喫煙による病態的変化の若年者と高齢者の比較

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長期間喫煙の病態に及ぼす影響について, 若年および高齢者喘息症例を対象に比較検討した。1. 吸入抗原に対する特異的IgE抗体の陽性率は, 高齢者では喫煙例において非喫煙例に比べ有意に高い値を示したが, 若年例では両群間に有意の差は見られなかった。2. %FVCやFEV_{1.0%}値は, 若年齢と高齢者例の間には有意差が見られたが, 喫煙例と非喫煙例の間には有意の差は見られなかった。3. %DLco値は若年, 高齢者例とも喫煙例で非喫煙例と比べ有意に低い値を示した。4. 肺の過膨張を示す%RVや%LAAは, 若年例に比べ

高齢者喘息で, また高齢者喘息では非喫煙例に比べ喫煙例で有意に高い値を示したが, %RVは若年例では喫煙例と非喫煙例の間に有意の差は見られなかった。5. 気道過敏性は, 高齢者喘息に比べ若年例で, また高齢者喘息では非喫煙例に比べ喫煙例でより高度であったが, 若年例では喫煙例と非喫煙例の間に有意の差は見られなかった。6. LTB₄, LTC₄の産生はいずれも高齢者喘息では, 非喫煙例に比べ喫煙例で有意の亢進が見られた。以上の結果より, 非喫煙例と喫煙例の比較では, %DLco, LTC₄産生および%LAAでは, 若年例, 高齢者喘息いずれにおいても, またIgE抗体産生, %RV, 気道過敏性およびLTB₄産生は高齢者喘息においてのみ, 有意の差が見られたこと, 一方, %FVC, FEV_{1.0%}などの換気機能検査では非喫煙例, 喫煙例の間に有意の差は見られないことが示された。さらに, 若年例と高齢者例との間では, %DLcoおよびLTC₄産生以外は全て有意の差が見られた。